

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
30 November 2000 (30.11.2000)

PCT

(10) International Publication Number
WO 00/71754 A1

- (51) International Patent Classification⁷: C12Q 1/68, 07078 (US). STENROOS, Edward, Scott [US/US]; 2nd floor, 317 Ann Street, Harrison, NJ 07029 (US).
C07K 14/47, C12N 15/85
- (21) International Application Number: PCT/US00/14354 (74) Agent: DAVIS, Michael, D.; Klauber & Jackson, 411 Hackensack Avenue, Hackensack, NJ 07601 (US).
- (22) International Filing Date: 24 May 2000 (24.05.2000) (81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data: 09/318,448 25 May 1999 (25.05.1999) US (84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).
- (63) Related by continuation (CON) or continuation-in-part (CIP) to earlier application: US 09/318,448 (CON) Filed on 25 May 1999 (25.05.1999)
- (71) Applicant (*for all designated States except US*): UNIVERSITY OF MEDICINE AND DENTISTRY OF NEW JERSEY [US/US]; Suite 3200, 335 George Street, P.O. Box 2688, New Brunswick, NJ 08903 (US).
- (72) Inventors; and
- (75) Inventors/Applicants (*for US only*): JOHNSON, William, G. [US/US]; 91 Stewart Road, Short Hills, NJ
- Published:**
— *With international search report.*
— *Before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments.*
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

WO 00/71754 A1

(54) Title: METHODS FOR DIAGNOSING, PREVENTING, AND TREATING DEVELOPMENTAL DISORDERS DUE TO A COMBINATION OF GENETIC AND ENVIRONMENTAL FACTORS

(57) Abstract: The present invention discloses a novel method for identifying an individual who may be susceptible to develop a developmental disorder. In one particular example, an individual is identified who is genetically susceptible to becoming schizophrenic. In addition, the present invention discloses a novel method for identifying individuals who are genetically susceptible to have offspring with a developmental disorder. Methods of diagnosing, preventing and treating developmental disorders such as schizophrenia are also provided.

**METHODS FOR DIAGNOSING, PREVENTING, AND TREATING
DEVELOPMENTAL DISORDERS DUE TO A COMBINATION OF
GENETIC AND ENVIRONMENTAL FACTORS**

FIELD OF THE INVENTION

- 5 The invention relates generally to novel methods of diagnosing, preventing, and treating specific diseases which are caused by a combination of genetic and environmental factors. One such disease exemplified is schizophrenia.

BACKGROUND OF THE INVENTION

- 10 The term "schizophrenia" was introduced by Bleuler in the beginning of this century to encompass a dissociation or disruption of thought processes, along with a dichotomy among thought, emotion, and behavior [Bleuler, *Translation J. Zinkin*, New York: International University Press (1950)]. The current definition of schizophrenia includes a break with reality that is usually manifested as hallucinations, delusions, or disruption in thought processes [Carpenter *et al.*, *Medical*
15 *Progress*, 330:681-690 (1994)]. At present the nationally accepted definition for the diagnosis of schizophrenia is contained in Diagnostic and Statistical Manual for Mental Disorders, Fourth Edition, Washington, D.C (1994): American Psychiatric Association, hereby incorporated by reference in its entirety.

- Schizophrenia is a clinical syndrome that has a profound influence on public health.
20 The symptoms for schizophrenia begin early in life, and continues for most patients throughout their lives. An estimate of the direct and indirect costs of schizophrenia was thirty-three billion dollars for 1990 in the United States alone [Carpenter *et al.*, 1994, *supra*]. Indeed, one of every forty dollars spent for total health care expenditures in the United States is spent on treating schizophrenia [Rupp *et al.*,
25 *Psychiatric Clin. North Am.*, 16:413-423 (1993)]. Furthermore, estimates have been made suggesting that up to 50% of the homeless American population is schizophrenic [Bachrach, In: *Treating the Homeless Mentally Ill*, Washington, D.C., American Psychiatric Press, 13-40, Lamb *et al.* ed. (1992)].

The genetic factors in schizophrenia, though clearly documented to be present, are not simple [Carpenter and Buchanan, *N. Engl. J. Med.*, 330:681-689 (1994); Gottesman, *Clin. Genet.*, 46:116-123 (1994)]. Schizophrenia is, at least in part, a neurodevelopmental disorder, a birth defect in which the brain has been subtly
5 damaged during development [Carpenter and Buchanan, *N. Engl. J. Med.*, 330:681-689 (1994); Weinberger, *Arch. Gen. Psychiatry*, 44:660-669 (1987); Brixey *et al.*, *J. Clin. Psychol.*, 49:447-456 (1993)]. Evidence of this damage is seen both at autopsy [Kovelman and Scheibel, *Biol. Psychiatry*, 19:1601-1621 (1984); Bogerts *et al.*, *Arch. Gen. Psychiatry*, 42:784-791 (1985); Jakob and Beckman, *J. Neural Transm.*,
10 65:303-326 (1986); Brown *et al.*, *Arch. Gen. Psychiatry*, 43:36-42 (1986); Benes and Bird, *Arch Gen Psychiatry*, 44:608-616 (1987); Colter *et al.*, *Arch Gen Psychiatry*, 44:1023 (1987); Altshuler *et al.*, *Arch. Gen. Psychiatry*, 47:1029-1034 (1990); Pakkenberg, *Schizophr. Res.*, 7:95-100 (1992); Bogerts, *Schizophr. Bull.*, 19:431-445 (1993); Shapiro, *Schizophr. Res.*, 10:187-239 (1993)] and by neuroimaging [Jeste *et al.*, *Br. J. Psychiatry*, 153:444-459 (1988); Suddath *et al.*, *Am. J. Psychiatry*,
15 146:464-472 (1989); Suddath *et al.*, *N. Engl. J. Med.*, 322:789-794 (1990); DeLisi *et al.*, *Biol. Psychiatry*, 29:159-175 (1991); Breier *et al.*, *Arch. Gen. Psychiatry*, 49:921-926 (1992); O'Callaghan *et al.*, *J. R. Soc. Med.*, 85:227-231 (1992); Bogerts *et al.*, *Biol. Psychiatry*, 33:236-246 (1993); Andreasen *et al.*, *Science*, 266:294-298 (1994)].
20 The pattern of this brain damage and the presence of minor congenital abnormalities point to an insult occurring during the second trimester of fetal development [Bracha *et al.*, *Biol. Psychiatry*, 30:719-725 (1991); Bracha *et al.*, *Am. J. Psychiatry*, 149:1355-1361 (1992); Green *et al.*, *Psychiatry Res.*, 53:119-127 (1994)].
Epidemiological studies have documented a season-of-birth effect by which
25 schizophrenics are more frequently born during winter and early spring than during other seasons [Boyd *et al.*, *Schizophr. Bull.*, 12:173-186 (1986); Kendell and Adams, *Br. J. Psychiatry*, 158:758-763 (1991); O'Callaghan *et al.*, *Br. J. Psychiatry*, 158:764-769 (1991)]. Also, individuals exposed to an influenza epidemic [Mednick *et al.*, *Arch. Gen. Psychiatry*, 45:189-192 (1988); Barr *et al.*, *Arch. Gen. Psychiatry*,
30 47:869-874 (1990); O'Callaghan *et al.*, *Lancet*, 337:1248-1250 (1991); Murray *et al.*, *J. Psychiatr. Res.*, 26:225-235 (1992); Adams *et al.*, *Br. J. Psychiatry*, 163:522-534 (1993)] or famine [Susser and Lin, *Arch. Gen. Psychiatry*, 49:983-988 (1992)] during their second trimester of fetal development have increased risk of later

developing schizophrenia, according to some studies but not others [Kendell, *Arch. Gen. Psychiatry*, 46:878-882 (1989); Crow and Done, *Br. J. Psychiatry*, 161:390-393 (1992)]. This has suggested that an environmental effect such as dietary deficiency, virus infection [Kirch, *Schizophr. Bull.*, 19:355-370 (1993)], vitamin deficiency, or
5 effect of cold weather may be acting during fetal development.

Linkage mapping studies in schizophrenia have been difficult. Recently, some studies [Straub *et al.*, *Nature Genet.*, 11:287-293 (1995); Schwab *et al.*, *Nature Genet.*, 11:325-327 (1995); Moises *et al.*, *Nature Genet.*, 11:321-324 (1995)] have supported a gene locus on chromosome 6 (6p24-22, near the HLA region) as having
10 an effect in schizophrenia; other studies gave little or no support to a marker in this region [Wang *et al.*, *Nature Genet.*, 10:41-46 (1995); Mowry *et al.*, *Nature Genet.*, 11:233-234 (1995); Gurling *et al.*, *Nature Genet.*, 11:234-235 (1995); Antonarakis *et al.*, *Nature Genet.*, 11:235-236 (1995)]. At best this locus appeared to be involved in only about 15-30% of families [Straub *et al.*, 1995, *supra*]. Also, some evidence for
15 loci on chromosomes 3 [Pulver *et al.*, *Am. J. Med. Genet.*, 60:252-260 (1995), 8 [Pulver *et al.*, *Am. J. Med. Genet.*, 60:252-260 (1995); Kendler *et al.*, *Am. J. Psych.* 153:1534-1540 (1996), 9 [Coon *et al.*, *Biol. Psychiatry*, 34:277-289 (1993); Moises *et al.*, *Nature Genet.*, 11:321-324 (1995)] and 22 [Coon *et al.*, *Am. J. Med. Genet.*, 54:72-79 (1994); Pulver *et al.*, *Am. J. Med. Genet.*, 54:3-43 (1994)] have been
20 reported. In addition, two polymorphic markers very close to the gene encoding dihydrofolate reductase (DHFR) on chromosome 5q, D5S76 and D5S39, gave very high lod scores (as high as 6.49, *i.e.* odds of about 3 million to one in favor of genetic linkage versus chance occurrence) in 7 British and Icelandic schizophrenia families studied [Schwab *et al.*, *Nat. Genet.* 11:325-327 (1997); Straub *et al.*, *Molec*
25 *Psychiatr.* 2:148-155 (1997)]. However, this result could not be confirmed in studies of numerous other families.

There could be several reasons for this difficulty. First, there may be more than one gene involved, (locus heterogeneity). Second, the genetic factor(s) may be common in the population (high disease allele frequency), thus diminishing the power of
30 linkage studies [Terwilliger and Ott, *Handbook of Human Genetic Linkage*, Baltimore: Johns Hopkins Univ. Pr., 181 (1994)]. Third, the correct genetic model

may be unknown [Owen, *Psychol. Med.*, 22:289-293 (1992)]. Any or all of these factors could diminish the power of a linkage study sufficiently to make success very difficult [Terwilliger and Ott, 1994, *supra*].

Thus the current (developmental) model for schizophrenia is that genetic and environmental factors cause brain damage in a fetus that later develops schizophrenia. However, the genetic and environmental factors have not been identified. Also, extensive linkage and association studies have failed to identify genes determining schizophrenia.

Indeed, schizophrenia appears to be just one of a family of developmental disorders whose cause has not been identified. Other such developmental disorders are defined by the Diagnostic and Statistical Manual for Mental Disorders, Fourth Edition, Washington, D.C (1994) and include: Tourette Syndrome which is identical to Tourette's Disorder and is a subcategory of Tic Disorders; Bipolar Disorder which is identical with Bipolar I Disorder or Bipolar II disorder; Autism which is identical with Autistic Disorder which is a subcategory of Pervasive Developmental Disorders; Conduct disorder which is a subcategory of Attention-Deficit and Disruptive Behavioral Disorders; Attention-Deficit Hyperactivity Disorder which is identical to Attention-Deficit/Hyperactivity Disorder and to Attention-Deficit/Hyperactivity Disorder NOS (not otherwise specified) which is also a subcategory of Attention-Deficit and Disruptive Behavioral Disorders; Obsessive-Compulsive Disorder which is a subtype of Anxiety Disorders; Chronic Multiple Tics Syndrome which is identical to Chronic Motor or Vocal Tic Disorder which is a subtype of Tic Disorders; and Learning Disorders.

In addition Spina bifida is a developmental disorder. Spina bifida is a form of neural tube defect in which neural elements (spinal nerves or spinal chord) or coverings of the brain and spinal chord (dura mater, arachnoid mater) herniate through a midline defect into a cystic cavity covered completely or partially by skin.

Therefore, there is a need for new methods of diagnosing individuals susceptible to developing a developmental disorder. In addition, there is a need for methods of

identifying individuals susceptible to having offspring that develop a developmental disorder. Finally, there is a need for a method of treating such susceptible individuals in order to prevent and/or ameliorate the symptoms due to and/or associated with the developmental disorder.

- 5 The citations of any reference herein should not be construed as an admission that such reference is available as "Prior Art" to the instant application.

SUMMARY OF THE INVENTION

- The present invention provides methods of diagnosing, preventing and/or treating specific developmental disorders. Towards this end the present invention provides
- 10 methods of identifying an individual as being genetically or environmentally susceptible for developing or having a developmental disorder or for having offspring that develop the developmental disorder. Such a developmental disorder can be schizophrenia, spina bifida cystica, Tourette's syndrome, bipolar illness, autism, conduct disorders, attention deficit disorder, obsessive compulsive disorder, chronic
- 15 multiple tic syndrome and learning disorders such as dyslexia. In addition, any of the methods provided herein for identifying an individual as being genetically and/or environmentally susceptible for having or developing a developmental disorder or for having offspring that develop the developmental disorder can also be used in diagnosing the individual, preferably in conjunction with a clinical diagnosis.
- 20 Therefore, the present invention provides methods of identifying an individual as being genetically susceptible for having or developing a developmental disorder. The present invention further provides methods of identifying an individual as being genetically susceptible for having offspring that are susceptible for developing a developmental disorder. Methods of identifying an individual as being susceptible
- 25 due to environmental factors for having or developing a developmental disorder are also provided. In addition, the present invention provides methods of identifying an individual as being susceptible of having offspring that are susceptible for developing a developmental disorder. The present invention also provides methods of identifying an individual as being susceptible for having or developing a developmental disorder

due to both environmental and genetic factors. The present invention further provides methods of identifying an individual as being susceptible for having offspring that are susceptible for developing a developmental disorder

5 The present invention therefore provides methods for compiling genetic reference datasets, environmental reference datasets and/or genetic and environmental reference datasets for use in determining a predicted probability for an individual of having a susceptibility for having or developing a developmental disorder, or for having offspring that develop a developmental disorder.

10 In one aspect of the invention, the present invention provides methods that comprise generating a genetic reference dataset for use in determining the predicted probability of an individual for having a susceptibility for having or developing a developmental disorder due to genetic factors, or for having offspring that develop a developmental disorder due to genetic factors.

15 One such embodiment comprises collecting a biological sample from a human subject. The human subject can be a diagnostic proband, a blood relative of the diagnostic proband, an affected proband, a blood relative of the affected proband, a control proband, and/or a blood relative of the control proband. The biological sample contains nucleic acids and/or proteins from the human subject. The nucleic acids and/or proteins from the biological sample are then analyzed resulting in a
20 partial or full genotype for the alleles of the genes involved in folate, pyridoxine, and/or cobalamin metabolism. The partial or full genotype then forms a dataset of genetic explanatory variables for the human subject. The dataset of genetic explanatory variables is then compiled from multiple human subjects into a genetic reference dataset. Such compilations are exemplified in the Detailed Description and
25 Examples below.

In another aspect, the present invention provides a method that comprises generating a genetic and environmental reference dataset for use in determining the predicted probability of an individual for having a susceptibility for having or developing a developmental disorder due to genetic factors and environmental factors, or for

having offspring that develop a developmental disorder due to genetic factors and environmental factors. One such embodiment comprises obtaining dietary and epidemiological information for environmental explanatory variables for the human subjects and combining the environmental explanatory variables with a genetic
5 reference dataset for the human subjects as described above.

In another aspect, the present invention provides an environmental reference dataset for use in the determination of the predicted probability for an individual for having a susceptibility for having or developing a developmental disorder due to environmental factors, or for having offspring that develop a developmental disorder
10 due to environmental factors. One such embodiment comprises obtaining dietary and epidemiological information for environmental explanatory variables for a human subject. The human subject can be a diagnostic proband, a blood relative of the diagnostic proband, an affected proband, a blood relative of the affected proband, a control proband, or a blood relative of the control proband. The dataset of
15 environmental explanatory variables is then compiled from multiple human subjects into an environmental reference dataset for the human subjects.

The developmental disorder forming the basis of the reference datasets of the present invention can be schizophrenia, or spina bifida cystica, or Tourette's syndrome, or dyslexia, or conduct disorder, or attention-deficit hyperactivity disorder, or bipolar
20 illness, or autism, or chronic multiple tic syndrome or obsessive-compulsive disorder, or like disorders. A blood relative is preferably the mother of the individual, a sibling, the father or a grandparent of the individual. When the reference dataset is for use in the determination of the predicted probability for an individual of having a susceptibility for having offspring that develop a developmental disorder, the
25 individual is preferably a pregnant woman. The reference datasets of the present invention are themselves part of the present invention.

The present invention further provides methods of estimating the genetic susceptibility of an individual to have or to develop a developmental disorder, or to have offspring that develop a developmental disorder. In one such embodiment the
30 method comprises collecting a biological sample from a participant (or participants)

who is either the individual or a blood relative of the individual. The biological sample contains nucleic acids and/or proteins of the participant. The analysis of the nucleic acids and/or proteins from the biological sample yield a partial or full genotype for the alleles of the genes involved in folate, pyridoxine, and/or cobalamin metabolism. The partial or full genotype forms a dataset of genetic explanatory variables for the participants. The dataset of genetic explanatory variables obtained are added to a genetic reference dataset forming a combined genetic dataset. A model is then formulated comprising the genetic explanatory variables obtained from the participants and the combined genetic dataset is analyzed. A predicted probability for the individual for having and/or developing a developmental disorder and/or having offspring that develop a developmental disorder is then determined. The genetic susceptibility of an individual to have or to develop a developmental disorder and/or have offspring that develop a developmental disorder is estimated. In a preferred embodiment, analyzing the combined genetic dataset is performed by binary linear regression. In a more preferred embodiment, the binary linear regression is performed with the SAS system. In another preferred embodiment, the model is modified by adding or subtracting one or more genetic explanatory variables and the combined genetic dataset is re-analyzed, preferably by binary logistic regression. In this case a model is chosen that best fits the data. This can be accomplished by testing the model for goodness of fit.

The present invention also provides methods of estimating the genetic and environmental susceptibility of an individual to have or to develop a developmental disorder and/or for having offspring that develop a developmental disorder. One such embodiment comprises collecting a biological sample from one or more participants. Again, the participant is either the individual or a blood relative of the individual. The biological sample contains nucleic acids and/or proteins of the participant. The nucleic acids and/or proteins from the biological sample are analyzed resulting in a partial or full genotype for the alleles of the genes involved in folate, pyridoxine, and/or cobalamin metabolism. The partial or full genotype forms a dataset of genetic explanatory variables for the participant. Dietary and epidemiological information for environmental explanatory variables for the participant(s) are also obtained which are used to form a dataset of environmental explanatory variables for the

participant(s). The datasets of genetic explanatory variables and the dataset of environmental explanatory variables are added to a genetic and environmental reference dataset forming a combined genetic and environmental dataset. A model is formulated comprising the genetic and environmental explanatory variables obtained from the participant(s). The combined genetic and environmental dataset is then analyzed and a predicted probability for the individual for having and/or developing a developmental disorder and/or for having offspring that develop a developmental disorder is determined. The genetic and environmental susceptibility of an individual to have or to develop a developmental disorder and/or have offspring that develop a developmental disorder is estimated. In a preferred embodiment, analyzing the combined genetic and environmental dataset is performed by binary linear regression. In a more preferred embodiment the binary linear regression is performed with the SAS system. In another preferred embodiment the model is modified by adding or subtracting one or more genetic and/or environmental explanatory variables and the combined genetic and environmental dataset is re-analyzed preferably, by binary logistic regression. In this case a model is chosen that best fits the data. This can be accomplished by testing the model for goodness of fit.

For any of these methods, the developmental disorder can be schizophrenia, spina bifida cystica, Tourette's syndrome, bipolar illness, autism, conduct disorder, attention deficit hyperactivity disorder, obsessive compulsive disorder, chronic multiple tic syndrome and learning disorders such as dyslexia.

In a particular embodiment, the individual is suspected of being genetically susceptible of having or for developing the developmental disorder and/or of being genetically susceptible of having offspring that develop the developmental disorder. In a preferred embodiment of this type, the individual is suspected of being genetically susceptible for having or for developing the developmental disorder and/or of being genetically susceptible of having offspring that develop the developmental disorder because a blood relative has the developmental disorder. In one such embodiment the blood relative is a parent, a sibling, or a grandparent. In a preferred embodiment the blood relative is the mother of the individual. In a particular embodiment in which the individual is suspected of being genetically

susceptible of having offspring that develop the developmental disorder, the individual is a pregnant woman. In another such embodiment the individual is the mate of the pregnant woman. In a particular embodiment exemplified below, the developmental disorder is schizophrenia.

- 5 Since the availability of the data regarding the genetic and environmental explanatory factors can vary in separate determinations, variations in the explanatory factors used is clearly envisioned by the present invention.

The present invention further provides methods of lowering the risk of a pregnant woman to have a child that will develop a developmental disorder. One such
10 embodiment comprises administering methylfolate, cobalamin or pyridoxine to the pregnant woman and/or fetus, which lowers the risk of the pregnant woman to give birth to a child with a developmental disorder. In a particular embodiment of this type, the pregnant woman had been previously determined to be susceptible of having offspring that develop a developmental disorder by a method disclosed herein. The
15 present invention further provides a method of determining if any treatment is advisable for a pregnant woman that is genetically susceptible to having offspring that develop a developmental disorder which comprises determining the concentration of a risk factor from a tissue sample or body fluid from the pregnant woman. When the concentration of the risk factor is statistically above or below an accepted normal
20 range, treatment is advisable.

The present invention further provides methods of determining if any treatment is advisable for a pregnant woman who has been determined to be susceptible to having offspring that develop a developmental disorder. One such embodiment comprises determining the concentration of a risk factor from a tissue sample or body fluid from
25 the pregnant woman. When the concentration of the risk factor is statistically above or below an accepted normal range, treatment is advisable. In a particular embodiment of this type, the pregnant woman had been previously determined to be susceptible of having offspring that develop a developmental disorder by a method disclosed herein.

Methods of monitoring the effect of the administration of methylfolate, cobalamin or pyridoxine to the pregnant woman who has been determined to be susceptible to having offspring that develop a developmental disorder are also included in the present invention. One such embodiment comprises determining the concentration of a risk factor from a tissue sample or body fluid from the pregnant woman. When the concentration of the risk factor is statistically within an accepted normal range, the treatment is deemed effective. In a particular embodiment of this type, the pregnant woman had been previously determined to be susceptible of having offspring that develop a developmental disorder by a method disclosed herein. The risk factor can be any substance and/or metabolite linked to folate and/or cobalamin and/or pyridoxine metabolism. In one embodiment, the risk factor is homocysteine. In yet another embodiment, the risk factor is folate. In still another embodiment, the risk factor is cobalamin.

The present invention also provides a method of treating an asymptomatic individual determined to be susceptible for developing a developmental disorder comprising administering methylfolate, cobalamin and/or pyridoxine. In a particular embodiment of this type, the asymptomatic individual had been previously determined to be susceptible of developing a developmental disorder by a method disclosed herein.

The DNA samples from the persons tested may be obtained from any source including blood, a tissue sample, amniotic fluid, a chorionic *villus* sampling, cerebrospinal fluid, and urine.

The present invention includes but is not limited to the examples of proteins encoded by genes involved in folate, cobalamin and pyridoxine metabolism compiled in Tables 2-7 in the Detailed Description of the Invention, below. For certain genes nucleic acid and/or amino acid sequence data is also provided. These genes and related sequence data are solely intended as examples of genes that are suitable to be used in the methods described herein. Such sequence data can be used for carrying out the genetic analysis of the present invention. However, the present invention is not intended to be limited in any way to such lists of proteins or the related sequence data.

It is further contemplated by the present invention to provide methods that include the testing for a genetic mutations in individual genes involved in folate and cobalamin metabolism and/or in individual combinations of such genes (*e.g.*, methylenetetrahydrofolate reductase gene and methionine synthase). In addition, all possible combinatorials, and permutations of such genes including a constellation comprising all of the genes involved in folate, pyridoxine, and cobalamin metabolism is envisioned by the present invention. Alternatively, a constellation of genes in which any one or more genes can be excluded from those tested is also contemplated by the present invention (for example, a given constellation of genes can include genes encoding all of the proteins in Table 2 and 4 except the folate receptor 2-like protein). Thus all of such possible constellations are envisioned by, and are therefore part of the present invention.

The present invention also provides DNA polymorphisms that can be used as genetic explanatory factors in the present invention. One such embodiment is a nucleic acid encoding a genetic variant of human dihydrofolate reductase comprising a nucleotide sequence having a 19 base-pair deletion spanning nucleotides 540 to 558 of the nucleotide sequence of SEQ ID NO:41. In a preferred embodiment the nucleic acid has the nucleotide sequence of SEQ ID NO:42.

The present invention also includes primers. One such embodiment is a PCR primer that can be used to distinguish SEQ ID NO:42 from SEQ ID NO:41. Another embodiment is a PCR primer that can be used to distinguish SEQ ID NO:42 from SEQ ID NO:45. These primers are useful for identifying the 19 base-pair deletion spanning nucleotides 540 to 558 of the nucleotide sequence of SEQ ID NO:41 (*see* Example 2). In a particular embodiment, the PCR primer comprises 8 to 100 and preferably 10 to 50 consecutive nucleotides from the nucleotide sequence of SEQ ID NO:41. In another embodiment the PCR primer comprises 8 to 100 and preferably 10 to 50 consecutive nucleotides from the nucleotide sequence of the complementary strand of SEQ ID NO:41. In still another embodiment the PCR primer comprises 8 to 100 and preferably 10 to 50 consecutive nucleotides from the nucleotide sequence of SEQ ID NO:42. In yet another embodiment the PCR primer comprises 8 to 100 and preferably 10 to 50 consecutive nucleotides from the nucleotide sequence of the

complementary strand of SEQ ID NO:42. In still another embodiment the PCR primer comprises 8 to 100 and preferably 10 to 50 consecutive nucleotides from the nucleotide sequence of SEQ ID NO:45. In yet another embodiment the PCR primer comprises 8 to 100 and preferably 10 to 50 consecutive nucleotides from the
5 nucleotide sequence of the complementary strand of SEQ ID NO:45.

In a particular embodiment the PCR primer comprises 8 to 100 and preferably 10 to 50 consecutive nucleotides from nucleotides 350 to 530 of SEQ ID NO:41. In a preferred embodiment of this type, the PCR primer has the nucleotide sequence of CTAAACTGCATCGTCGCTGTG (SEQ ID NO:38). In another particular
10 embodiment the PCR primer comprises 8 to 100 and preferably 10 to 50 consecutive nucleotides from the complementary strand of nucleotides 550 to 850 of SEQ ID NO:41. In preferred embodiment of this type, the PCR primer comprises 8 to 100 and preferably 10 to 50 consecutive nucleotides from the complementary strand of nucleotides 570 to 690 of SEQ ID NO:41. In a particular embodiment, the PCR
15 primer has the nucleotide sequence of AAAAGGGGAATCCAGTCGG (SEQ ID NO:39).

The present invention also provides a nucleic acid that hybridizes under standard hybridization conditions to the nucleotide sequence ACCTGGGCGGGACGCGCCA (SEQ ID NO:40). In another embodiment the nucleic acid hybridizes under standard
20 hybridization conditions to the nucleotide sequence complementary to SEQ ID NO:40. In yet another embodiment the nucleic acid hybridizes under standard hybridization conditions to the nucleotide sequence ACCTGGGCGGGACGCGCC (SEQ ID NO:46). In yet another embodiment the nucleic acid hybridizes under standard hybridization conditions to the nucleotide sequence complementary to SEQ
25 ID NO:46. In a particular embodiment the nucleic acid consists of 9 to 96 nucleotides. In another embodiment the nucleic acid consists of 12 to 48 nucleotides. In still another embodiment the nucleic acid consists of 15 to 36 nucleotides. In a preferred embodiment the nucleic acid consists of 17 to 20 nucleotides.

The present invention also provides a nucleic acid that hybridizes to the nucleotide
30 sequence of SEQ ID NO:41, but not to the nucleotide sequence of SEQ ID NO:42

when the hybridization is performed under identical conditions. In a particular embodiment the nucleic acid comprises the nucleotide sequence of CCCACGGTCGGGGTACCTGGGCGGGACGCGCCAGGCCGACTCCCGGCCGA (SEQ ID NO:29). The present invention further provides a nucleic acid that

5 hybridizes to the nucleotide sequence of SEQ ID NO:42, but not to the nucleotide sequence of SEQ ID NO:41 when the hybridization is performed under identical conditions. In a particular embodiment the nucleic acid comprises the nucleotide sequence of CCCACGGTCGGGGTGGCCGACTCCCGGCCGA (SEQ ID NO:37).

In a related embodiment the present invention provides an isolated nucleic acid that

10 hybridizes to the complementary strand of the nucleotide sequence of SEQ ID NO:42, but not to the complementary strand of the nucleotide sequence of SEQ ID NO:41 when the hybridization is performed under identical conditions. In still another embodiment the nucleic acid hybridizes to the nucleotide sequence of SEQ ID NO:41, but not to the nucleotide sequence of SEQ ID NO:42 when the hybridization

15 is performed under identical conditions. In still another embodiment the nucleic acid hybridizes to the complementary strand of the nucleotide sequence of SEQ ID NO:41, but not to the complementary strand of the nucleotide sequence of SEQ ID NO:42 when the hybridization is performed under identical conditions.

The present invention also provides a nucleic acid that hybridizes to the nucleotide

20 sequence of SEQ ID NO:42, but not to the nucleotide sequence of SEQ ID NO:45 when the hybridization is performed under identical conditions. In a related embodiment the present invention provides an isolated nucleic acid that hybridizes to the complementary strand of the nucleotide sequence of SEQ ID NO:42, but not to the complementary strand of the nucleotide sequence of SEQ ID NO:45, when the

25 hybridization is performed under identical conditions. In still another embodiment the nucleic acid hybridizes to the nucleotide sequence of SEQ ID NO:45, but not to the nucleotide sequence of SEQ ID NO:42 when the hybridization is performed under identical conditions. In still another embodiment the nucleic acid hybridizes to the complementary strand of the nucleotide sequence of SEQ ID NO:45, but not to the

30 complementary strand of the nucleotide sequence of SEQ ID NO:42 when the hybridization is performed under identical conditions.

The present invention also provides for the use of the nucleic acids of the present invention (as well as other nucleic acids which can be used to identify DNA polymorphisms in the alleles of the genes involved in folate, pyridoxine, and/or cobalamin metabolism) in the methods of the present invention for identifying,
5 diagnosing, preventing and/or treating individuals.

In methods of estimating the susceptibility due to genetic or genetic and environmental factors for an individual to have or to develop a developmental disorder or to have offspring that develop a developmental disorder, and for the corresponding methods of generating genetic, or genetic and environmental reference
10 datasets, the present invention provides a step of analyzing nucleic acids and/or proteins from biological samples. In one particular embodiment, the assaying for the presence of the genetic variant of human dihydrofolate reductase having a nucleotide sequence with a 19 base-pair deletion spanning nucleotides 540 to 558 of the nucleotide sequence of SEQ ID NO:41 is included as part of this analysis. This
15 genetic variant of human dihydrofolate reductase becomes a genetic explanatory variable.

Determining if the biological sample contains the genetic variant of human dihydrofolate reductase having a nucleotide sequence with a 19 base-pair deletion spanning nucleotides 540 to 558 of the nucleotide sequence of SEQ ID NO:41 can be
20 performed by any appropriate method including PCR, special PCR, RT PCR, RFLP analysis, SSCP, and FISH.

In addition, all of the nucleic acids of the present invention including cDNA or genomic DNA can be placed into expression vectors operably associated with an expression control sequence. Alternatively, when the nucleic acid is part of an
25 expression control sequence, the nucleic acid and/or the expression control sequence can be placed into an expression vector to control the expression of a coding sequence, such as a reporter gene. Such expression vectors can then be placed into either eukaryotic or prokaryotic host cells and expressed. The host cells comprising the expression vectors are also part of the present invention. In addition, when the
30 nucleic acid includes a coding sequence or a part of a coding sequence, the present

invention includes methods of purifying the gene products from the coding sequence or part thereof, and the purified gene products themselves.

Accordingly, it is a principal object of the present invention to provide a method for identifying an individual that is genetically inclined to develop a developmental
5 disorder or disease.

It is a further object of the present invention to provide a method for identifying an individual that is genetically inclined to develop schizophrenia.

It is a further object of the present invention to provide a method for identifying an individual that is genetically inclined to have offspring having a developmental
10 disorder.

It is a further object of the present invention to provide a method of diagnosing schizophrenia.

It is a further object of the present invention to provide a method of treating developmental disorders such as schizophrenia.

15 It is a further object of the present invention to provide a method for monitoring the treatment of the developmental disorder.

It is a further object of the present invention to provide a method for ameliorating the effect of a defect in folate, pyridoxine or cobalamin metabolism on a fetus due to the genetic or environmental status of a pregnant woman.

20 It is a further object of the present invention to provide a method of treating a patient who is genetically inclined to develop a developmental disorder such as schizophrenia.

It is a further object of the present invention to provide a method of overcoming a nutritional lack of folate, cobalamin or pyridoxine of a pregnant woman to prevent the development of the corresponding fetus developing a developmental disorder.

Other objects and advantages will become apparent to those skilled in the art from a
5 review of the ensuing description.

These and other aspects of the present invention will be better appreciated by reference to the following drawings and Detailed Description.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows primers for PCR amplification of the dihydrofolate reductase (DHFR)
10 deletion polymorphism region.

Figure 2 shows the genotypes of the DHFR 19 basepair deletion by non-denaturing polyacrylamide gel electrophoresis. Lanes 1 and 2 show genotypes 1,1. Lanes 3 and 4 show genotypes 1, 2. Lanes 5 and 6 show genotypes 2,2. Lane 7 shows phiX174 RF DNA/HaeIII size markers from BRL Life Technologies.

15 Figure 3 shows the sequences of PCR amplification products in the Region of the DHFR polymorphism region. * is explained in Text, see Example 2.

Figure 4A is a nucleotide sequence of the wild type human DHFR, (SEQ ID NO:41) from Yang *et al.*, *J. Mol. Biol.* 176:169-187 (1984), GeneBank accession no: X00855. The start codon is in bold. Figure 4B is the same nucleotide sequence as that of

20 Figure 4A except the deletion of the 19 nucleotides due to the DHFR deletion polymorphism, (SEQ ID NO:42).

DETAILED DESCRIPTION OF THE INVENTION

The present invention in its broadest embodiment provides a method of diagnosing, preventing and/or treating specific physiological/developmental disorders. Such physiological/developmental disorders include schizophrenia, spina bifida cystica, 5 Tourette's syndrome, bipolar illness, autism, conduct disorders, attention deficit disorder, obsessive compulsive disorder, chronic multiple tic syndrome and learning disorders such as dyslexia.

A particular aspect of the present invention provides methodology for diagnosing, preventing and/or treating a developmental disorder such as schizophrenia. Such 10 methodology is premised on the correlation between abnormalities in folate, cobalamin, and/or pyridoxine metabolism in an individual and/or the mother of an individual and the occurrence of the developmental disorder, *e.g.*, schizophrenia in the individual. Further, the present invention provides a framework (*i.e.*, the gene-teratogen model, and the DNA Polymorphism-Diet-Cofactor-Development both of 15 which are described in detail below) which fully explain the rationale for the correlation. though the ultimate usefulness of the methods of the present invention are independent of any particular model.

Within this context, the DNA Polymorphism-Diet-Cofactor-Development model maintains that a developmental disorder such as schizophrenia results in part from 20 developmental brain damage sustained *in utero* due to maternal dietary deficiency of folate, pyridoxine or cobalamin potentiated by the aggregate effect of minor defects of folate, pyridoxine or cobalamin genes. The maternal damage to the fetus can result in part from insufficiency of the folate, pyridoxine and cobalamin themselves and/or from resulting effects such as immune deficiency and maternal teratogens, *e.g.* 25 hyperhomocysteinemia. Genes from either parent acting in the fetus may modify these damaging effects as exemplified in the gene-teratogen model, below.

As described herein the present invention can be practiced on a case by case basis, or alternatively, it can be used in the screening of the general population, or within any

particular subgroup, such as newborns (as is presently performed in the diagnosis and treatment of hyperphenylalaninemia).

Therefore, if appearing herein, the following terms shall have the definitions set out below.

- 5 As used herein a "gene involved in folate, pyridoxine, or cobalamin metabolism" is a gene that encodes a peptide or protein that plays a role in a pathway involved in either folate, pyridoxine, or cobalamin metabolism. An incomplete listing of examples of such proteins is given in Tables 2-7.

- As used herein the term "individual" includes a fetus, infant, child, adolescent, and
10 adult. Therefore, as used herein, an individual originates at conception.

As used herein an individual with a susceptibility for "having offspring that develop a developmental disorder" is meant to be indicative of the susceptibility of the offspring of that individual to develop the developmental disorder and is not in any way meant to be indicative of the susceptibility of the individual to have offspring.

- 15 The term "proband" as used herein is operationally defined by Table 8 along with the accompanying explanatory information (*see*, Example 1). For most purposes, the proband can be considered the central figure in the familial analysis, the remaining individuals in the family being designated as "blood relatives". There are three types of probands: (1) an "affected proband" *i.e.*, an individual that is believed to have a
20 developmental disorder ; (2) a "control proband" an individual that is believed not to have a developmental disorder; and (3) a "diagnostic proband" *i.e.*, an individual being diagnosed.

- As used herein a "blood relative" of an individual is a relative that is related to the individual in a genetic sense. Blood relatives can include mothers, fathers, children,
25 uncles, aunts, brothers, sisters, and grandparents. Preferably a blood relative is a parent, a sibling, or a grandparent. Adopted relatives, step-parents, relatives through marriage and the like are not blood relatives. Therefore, as used herein, the terms

“mother”, “father”, “sibling”, “grandparent”, “grandfather” and “grandmother” are indicative of blood relationships.

As used herein a “mate of an individual” is a person whose genetic material is combined with that of the individual for the conception of the offspring in question.

- 5 As used herein the term “schizophrenia” describes a disorder that is at least partially due to one or more genetic mutations or polymorphisms in one or more genes involved in folate, cobalamin or pyridoxine metabolism in an individual that is schizophrenic and/or to one or more genetic mutations or polymorphisms in one or more genes involved in folate, cobalamin or pyridoxine metabolism in the mother of
10 that individual.

- As used herein an individual is “schizophrenic” when the individual displays symptoms that would be accepted by an experienced psychiatrist to merit a diagnosis of schizophrenia. Such a diagnosis is based, at least in part, on the currently evolving guidelines for the diagnosis of schizophrenia which are listed in the successive
15 editions of Diagnostic and Statistical Manual for Mental Disorders, put out by the American Psychiatric Association. The current edition is the DSM, Fourth Edition (1994).

- As used herein the terms “spina bifida cystica”, “Tourette’s syndrome”, “bipolar illness”, “autism”, “conduct disorder”, “attention deficit disorder”, “obsessive
20 compulsive disorder”, “chronic multiple tic syndrome” and “learning disorders” such as “dyslexia” describe disorders which display symptoms that would be accepted by an experienced psychiatrist to merit a diagnosis of that disorder. Such a diagnosis is based, at least in part, on the currently evolving guidelines which are listed in the successive editions of Diagnostic and Statistical Manual for Mental Disorders, put out
25 by the American Psychiatric Association. The current edition is the DSM, Fourth Edition (1994).

As used herein the term “teratogenic locus” indicates one or more alleles that act in a pregnant woman to cause an intrauterine teratogenic effect on the fetus.

As used herein the terms "specificity locus" or "modifying locus" are used interchangeably and are indicative of one or more alleles that can act during pregnancy and/or after birth to prevent, modify, and/or ameliorate the teratogenic effect of the teratogenic locus.

- 5 As used herein a "constellation of genetic mutations" is the set of genetic risk factor mutations that is present in a proband and relatives of the proband. One example of a constellation of genetic mutations is shown in a line of Table 8, below.

- As used herein a "risk factor" is a teratogen or substance (including a defective gene) that can lead to a teratogenic effect that is present or suspected of being present in a
10 tissue sample or body fluid of an individual's mother during the individual's gestation and/or present or suspected of being present in a tissue sample or body fluid of the individual.

- As used herein a "genetic risk factor" is used interchangeably with the term "genetic explanatory variable" and is a genetic mutation and/or polymorphism that causes or
15 potentially can cause the formation of and/or lead to the development of a risk factor in an individual or the individual's mother during gestation.

- As used herein an "environmental risk factor" is used interchangeably with the term "environmental explanatory variable" and is an environmental factor that causes or
20 potentially can cause the formation of and/or lead to the development of a risk factor in an individual or the individual's mother during gestation.

As used herein an "explanatory variable" is either an "environmental explanatory variable" or a "genetic explanatory variable" or the variable defined by their interaction or any combination of the above.

- Enzymes whose deficiency may raise plasma homocysteine include
25 methylenetetrahydrofolate reductase (MTHFR), methionine synthase, and folate receptors/transport proteins/binding proteins (as well as all of the proteins listed in Tables 2-7 below).

- The current (developmental) model for schizophrenia is that genetic and environmental factors cause brain damage in a fetus that later develops schizophrenia. However, the genetic and environmental factors have not been identified. Also, extensive linkage and association studies have failed to identify genes determining schizophrenia. The reasons usually given for this difficulty include: (i) locus heterogeneity, *i.e.*, more than one gene locus is involved, perhaps many gene loci each with a small effect; (ii) the mode of inheritance of schizophrenia is unknown; and (iii) an additional possible factor is that the frequency of the disease alleles may be high, thus greatly reducing the power of linkage studies.
- 10 The DNA Polymorphism-Diet-Cofactor-Development model explains all of these difficulties and at the same time proposes a unified metabolic abnormality. The unified metabolic abnormality is: (a) ENVIRONMENTAL, *i.e.*, due to a folate/cobalamin/pyridoxine deficiency caused by either decreased ingestion or increased requirement during pregnancy; (b) GENETIC, *i.e.*, due to a
- 15 folate/cobalamin/pyridoxine genetic defect caused by the aggregate effect of multiple mutations of folate/cobalamin/pyridoxine genes each individually having a small effect; and (c) the interaction of the folate/cobalamin/pyridoxine environmental and genetic factors (indicated above) to cause other harmful effects such as maternal teratogens and immune deficiency during gestational development. Different gene
- 20 loci and different combinations of gene loci will be involved in different patients and different families. The problem of locus heterogeneity is addressed by the hypothesis that the folate/cobalamin/pyridoxine genetic defect is the aggregate effect of multiple mutations of folate/cobalamin/pyridoxine genes each of which have a relatively small effect.
- 25 The problem of mode of inheritance is addressed by the gene-teratogen model. The gene-teratogen model describes the special features of genes acting *in utero*; both teratogenic and modifying of specificity loci may be involved. If these effects are not taken into account, the assignment of affection status in schizophrenia pedigrees is inaccurate. Assignment of affection status is a key element in defining the mode of
- 30 inheritance for all kinds of linkage mapping. Failure to assign the correct mode of inheritance is another factor that has made the linkage studies very difficult.

Finally, the DNA Polymorphism-Diet-Cofactor-Development model proposes that some of the genetic factors for schizophrenia are common in the population. In fact, subclinical deficiency of folate, pyridoxine, and cobalamin is common in the population and common among pregnant women as well. Pregnancy further
5 increases the requirement for folate, pyridoxine, and cobalamin. Common genetic polymorphisms of folate and cobalamin genes are also known, some of them functional. Common genetic risk factors tend to be functional polymorphisms and/or mutant alleles that individually have small effects. Otherwise, they would be largely eliminated from the population by natural selection and would not be common. High
10 disease allele frequency is yet another factor that greatly diminishes the power of a linkage study.

Besides explaining the difficulties with current linkage studies, the DNA Polymorphism-Diet-Cofactor-Development model explains all of the unusual biological and epidemiological features of schizophrenia: *e.g.* the decreased amount
15 of gray matter in brain areas, the unusual birth-month effect, the geographical differences in incidence, the socioeconomic predilection, the association with obstetrical abnormalities (low birth weight and prematurity), and the association with famine and viral epidemics. Consistently, genetic linkage and cytogenetic studies in schizophrenia have implicated various chromosome regions, some of them containing
20 folate, pyridoxine, and cobalamin genes including dihydrofolate reductase, thymidylate synthase, and transcobalamin II. The DNA Polymorphism-Diet-Cofactor-Development model predicts that folate, pyridoxine, or cobalamin gene mutations have a high frequency in schizophrenia patients or family members. Furthermore, mothers of schizophrenics are predicted to be particularly
25 susceptible to producing one or more teratogens during pregnancy.

The present invention therefore provides methods for: (a) Diagnostic testing of schizophrenia by identifying a folate, pyridoxine, or cobalamin gene mutation or constellation of mutations in the patient, mother, and father. (b) Prevention of schizophrenia by diagnostic testing in families already affected by schizophrenia or
30 by diagnostic population screening for folate mutations and identifying couples at risk for producing schizophrenic offspring. These pregnancies can be further monitored

- for risk factors, *e.g.* dietary folate/pyridoxine/cobalamin, plasma folate/pyridoxine/cobalamin, or red blood cell folate; plasma homocysteine or other teratogens. (c) Therapy for schizophrenia, *e.g.*, treating the pregnant mother with folate, pyridoxine, cobalamin or other agents. The treatment can be monitored at
- 5 regular intervals to determine the effect of therapy. (d) Presymptomatic treatment of schizophrenia on young children found to be susceptible to schizophrenia by diagnostic testing for folate gene mutations and other risk factors can also be treated with methylfolate or related therapeutic modalities to forestall the appearance of schizophrenia symptoms in adolescence or adulthood.
- 10 Empirical studies with methylfolate treatment of schizophrenia have shown modest clinical improvement. The DNA Polymorphism-Diet-Cofactor-Development model gives a rationale for such therapy as well as for intensive testing of related therapeutic modalities. Genetic testing will need to be carried out in such patients to gauge their likelihood of responding to therapy. In addition, the DNA
- 15 Polymorphism-Diet-Cofactor-Development model gives direction and impetus toward uncovering the mechanism of fetal brain damage leading to schizophrenia.

- Diagnostic testing for schizophrenia can involve testing not just the patient, but mother and father as well, for not just one factor but multiple genetic factors. For example, data for two gene loci (both folate-related genes) were used in Example 2.
- 20 In this case, there were only four explanatory variables for each comparison.

- In addition, risk factors appearing only during pregnancy may play a role, *e.g.* dietary folate which can be further monitored during the pregnancy. In certain instances, genotype data can be used as the sole explanatory variables, particularly in the case when no environmental explanatory variables are known. In such a case, the
- 25 predicted probabilities will be only for the genetic component of the proband's risk of schizophrenia. In addition, schizophrenia mothers, fathers, and sibs do not necessarily have to come from the same families as the schizophrenia probands, as described in Example 2.

Of course certain genetic factors will turn out to be more common than others. This may simplify testing somewhat. Also some genetic factors may operate chiefly in the mother, while others will operate chiefly in the schizophrenic patient. This may also simplify testing. There are some approaches to assessing risk factors during a past pregnancy, *e.g.* current dietary history as an indicator of past diet, methionine loading as an indicator of how susceptible a mother is to raising her plasma homocysteine, assessment of other risk factors besides folate metabolism that may affect pregnancy outcome. Procedures including all of these variables are both envisioned and included in the present invention.

- 10 Thus the present invention provides a method of diagnosis of schizophrenia. In one aspect of the invention, diagnostic testing for genetic susceptibility to schizophrenia determines the probability that the proband is affected with schizophrenia due to genetic factors. This is carried out by genetic testing of a patient suspected of having schizophrenia and/or whatever informative relatives are available, *e.g.* mother, father, 15 sibs, or children. The genotypes of certain folate and/or cobalamin and/or pyridoxine gene mutations or constellation of mutations (folate and/or cobalamin and/or pyridoxine gene mutations) are determined for each individual.

Since the abnormal phenotype of schizophrenia can be determined by both genetic and environmental factors and since other genetic factors besides 20 folate/cobalamin/pyridoxine gene mutations may be involved, the presence of folate/cobalamin/pyridoxine gene mutations may be neither necessary nor sufficient to cause schizophrenia. Thus, an unaffected individual may have the same genetic risk factors as an affected individual but may lack sufficient environmental factors to cause the abnormal clinical disease. Also, an affected individual may lack 25 folate/cobalamin/pyridoxine gene mutations but may have other related or non-related genetic risk factors that caused the schizophrenia.

Therefore folate/cobalamin/pyridoxine gene mutations are used as explanatory variables (genetic risk factors) to calculate the predicted probability that an individual has genetic susceptibility to schizophrenia due to these mutations. Genetic variation 30 can be expected to account for approximately about half of the risk of developing

schizophrenia since the concordance rate in identical twins has been estimated to be about 50%. The other half of the risk results from environmental factors due to their different positions in the uterus and to differences in the blood supply. The use of environmental factors as additional explanatory variables enhances this probability calculation, although this environmental data is more difficult to gather. Together, using both genetic and environmental explanatory variables, the predicted probability that an individual is schizophrenic may approach 1.0.

One likely situation for the use of the present methodology is in the diagnosis of a patient that has developed a psychosis. In such a case, the clinician is likely to be interested in determining the probability that this individual has schizophrenia. The number of blood relatives (preferably first degree relatives) of the patient-to-be diagnosed, both unaffected and affected, could then be determined. The number of these who would contribute a blood sample for analysis, for example, could then be ascertained. It is preferable that the patient-to-be-diagnosed also contributes a blood sample, however in certain situations, this may not be an option. The availability of dietary and epidemiological information for environmental explanatory variables, especially from the patient and the mother, can also be ascertained. Of course all relevant legal and ethical rules should be followed regarding informed consent for the genetic testing.

Biological samples such as tissue or fluid samples (*e.g.*, 7 ml of blood in an EDTA-containing vacutainer, *see* Example 2, below), and obtainable environmental data from the patient and family members are then collected. DNA is extracted from the sample and genotypes for alleles of folate and/or cobalamin and/or pyridoxine genes are determined. The methods for genotyping depend upon the specific genetic markers used as explanatory variables. The methods for allele determination for two genetic markers are discussed in the Examples below.

Data of the genetic and environmental explanatory variables for the patient-to-be-diagnosed (proband) and participating family members are added to a reference data set preferably consisting of well-defined schizophrenia probands and family members, and control probands, and family members for whom data is

available for many explanatory variables. As an approximation the control probands themselves also can be used as the controls for each proband family member class as shown in Example 2, below. Thus, as an approximation the control probands can be used as controls for the affected probands; and/or separately for the mothers of
5 affected probands; and/or separately for the fathers of affected probands, etc. Another example of a use of the control probands is in the evaluation and/or analysis of a particular diagnostic proband. In this case, the approximation is obtained by adding the diagnostic proband to the group of affected probands and control probands.

A model is then created consisting of the explanatory variables actually available
10 from specific patient-to-be diagnosed and family members participating in the testing. This new combined data set (reference data set and data from patient-to-be-diagnosed with participating family members) is analyzed by binary logistic regression (e.g., using a statistical software package such as the SAS System embodied in Example 1 below, though other programs may be used) for the model chosen giving the
15 predicted probability that a proband is affected with schizophrenia for all of the probands including the patient-to-be-diagnosed.

In a particular embodiment the model is modified and the goodness of fit for the patient-to-be-diagnosed is checked. The predicted probability that the patient-to-be-diagnosed has schizophrenia is compared with a classification table
20 generated from the model used to determine the likelihood of false positives and false negatives.

The predicted probability that the patient-to-be-diagnosed is affected with schizophrenia, with the likelihood of false positive or false negative result, can then be forwarded to the clinician.

25 The methods for determining an individual's risk for developing schizophrenia taught by the present invention can be used in a variety of settings. For example, the present invention also provides a therapy for schizophrenia. Empirical studies with methylfolate treatment of schizophrenia have shown modest clinical improvement. The DNA Polymorphism-Diet-Cofactor-Development model provides a rationale for

such therapy as well as for intensive testing of related therapeutic modalities, *e.g.* other cofactors such as cobalamin or pyridoxine. In addition, the DNA Polymorphism-Diet-Cofactor-Development model gives direction and impetus toward uncovering the mechanism of fetal brain damage leading to schizophrenia. Of course such therapy also can be provided on a case by case basis in order to gauge the likelihood of the patient of responding to such therapy, with the methodology for diagnosis of the present invention enabling the skilled practitioner to assess that likelihood.

In addition, the present invention provides a method of identifying individuals that are likely to be aided by presymptomatic treatment for schizophrenia. For example, young children found to have a high risk for susceptibility to schizophrenia by diagnostic testing can be treated with methylfolate or related therapeutic modalities to forestall the appearance of schizophrenia symptoms in adolescence or adulthood. The present invention further provides methodology for diagnostic testing for specific families already affected by schizophrenia.

The present invention further provides methodology for population screening for folate/cobalamin/pyridoxine mutations to help identify couples at risk for producing schizophrenic offspring. Subsequent or concurrent pregnancies can then be monitored for environmental risk factors, and treated with folate, cobalamin, pyridoxine or other agents and monitored at intervals for the effect of therapy. Such monitoring can include measuring levels of folate, cobalamin, pyridoxine or homocysteine in a particular tissue and/or fluid sample, such as blood.

Since schizophrenia is a developmental disorder, it is likely that these same risk factors discussed here for schizophrenia could play a role in other developmental disorders including spina bifida cystica, Tourette's syndrome, learning disorders including dyslexia, conduct disorder, attention-deficit hyperactivity disorder, bipolar illness, autism, and obsessive-compulsive disorder. Interestingly, the mode of inheritance of these disorders, like that of schizophrenia, has been difficult to determine despite the fact that a genetic component to the etiology of each has been documented. Therefore, methodology analogous to that exemplified herein for

schizophrenia can be readily adapted for diagnosing and/or treating other such developmental disorders.

Nucleic Acids

In accordance with the present invention there may be employed conventional
5 molecular biology, microbiology, and recombinant DNA techniques within the skill
of the art. Such techniques are explained fully in the literature. See, *e.g.*, Sambrook,
Fritsch & Maniatis, *Molecular Cloning: A Laboratory Manual*, Second Edition
(1989) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (herein
"Sambrook *et al.*, 1989"); *DNA Cloning: A Practical Approach*, Volumes I and II
10 (D.N. Glover ed. 1985); *Oligonucleotide Synthesis* (M.J. Gait ed. 1984); *Nucleic Acid
Hybridization* [B.D. Hames & S.J. Higgins eds. (1985)]; *Transcription And
Translation* [B.D. Hames & S.J. Higgins, eds. (1984)]; *Animal Cell Culture* [R.I.
Freshney, ed. (1986)]; *Immobilized Cells And Enzymes* [IRL Press, (1986)];
B. Perbal, *A Practical Guide To Molecular Cloning* (1984); F.M. Ausubel *et al.*
15 (eds.), *Current Protocols in Molecular Biology*, John Wiley & Sons, Inc. (1994)].

A "nucleic acid molecule" refers to the phosphate ester polymeric form of
ribonucleosides (adenosine, guanosine, uridine or cytidine; "RNA molecules") or
deoxyribonucleosides (deoxyadenosine, deoxyguanosine, deoxythymidine, or
deoxycytidine; "DNA molecules"), or any phosphoester analogs thereof, such as
20 phosphorothioates and thioesters, in either single stranded form, or a double-stranded
helix. Double stranded DNA-DNA, DNA-RNA and RNA-RNA helices are possible.
The term nucleic acid molecule, and in particular DNA or RNA molecule, refers only
to the primary and secondary structure of the molecule, and does not limit it to any
particular tertiary forms. Thus, this term includes double-stranded DNA found, *inter*
25 *alia*, in linear or circular DNA molecules including restriction fragments, plasmids,
and chromosomes. In discussing the structure of particular double-stranded DNA
molecules, sequences may be described herein according to the normal convention of
giving only the sequence in the 5' to 3' direction along the nontranscribed strand of
DNA (*i.e.*, the strand having a sequence homologous to the mRNA). A "recombinant

DNA molecule" is a DNA molecule that has undergone a molecular biological manipulation.

A nucleic acid molecule is "hybridizable" to another nucleic acid molecule, such as a cDNA, genomic DNA, or RNA, when a single stranded form of the nucleic acid molecule can anneal to the other nucleic acid molecule under the appropriate conditions of temperature and solution ionic strength (*see* Sambrook *et al.*, *supra*). The conditions of temperature and ionic strength determine the "stringency" of the hybridization. High stringency hybridization conditions correspond to 50% formamide, 5x or 6x SSC. Hybridization requires that the two nucleic acids contain complementary sequences, although depending on the stringency of the hybridization, mismatches between bases are possible. The appropriate stringency for hybridizing nucleic acids depends on the length of the nucleic acids, the GC percentage, and the degree of complementation, variables well known in the art. The greater the degree of similarity or homology between two nucleotide sequences, the greater the value of T_m for hybrids of nucleic acids having those sequences. The relative stability (corresponding to higher T_m) of nucleic acid hybridizations decreases in the following order: RNA:RNA, DNA:RNA, DNA:DNA. For hybrids of greater than 100 nucleotides in length, equations for calculating T_m have been derived (*see* Sambrook *et al.*, *supra*, 9.50-10.51). For hybridization with shorter nucleic acids, *i.e.*, oligonucleotides, the position of mismatches becomes more important, and the length of the oligonucleotide determines its specificity (*see* Sambrook *et al.*, *supra*, 11.7-11.8). Preferably a minimum length for a hybridizable nucleic acid (e.g., a nucleotide probe or primer such as a PCR or RT-PCR primer) is at least about 12 nucleotides; preferably at least about 18 nucleotides; and more preferably the length is at least about 27 nucleotides; and most preferably at least about 36 nucleotides. Specific probes and primers that can be used to distinguish specific variants of the nucleic acids encoding the proteins involved in folate, pyridoxine, and/or cobalamin metabolism are also part of the present invention.

Such nucleotide probes and primers can be labeled or used to label complementary DNA (where appropriate) by any number of ways well known in the art including

using a radioactive label, such as ^3H , ^{14}C , ^{32}P , or ^{35}S , a fluorescent label, a boron label [U.S. Patent No: 5,595,878, Issued January 21, 1997 and U.S. Patent No: 5,876,938, Issued March 2, 1999 which are incorporated by reference in their entireties], and enzymatic tags such as urease, alkaline phosphatase or peroxidase. In the case of
5 enzyme tags, colorimetric indicator substrates are known which can be employed to provide a means visible to the human eye or spectrophotometrically, to identify specific hybridization with complementary nucleic acid-containing samples.

In a specific embodiment, the term "standard hybridization conditions" refers to a T_m of 55°C , and utilizes conditions as set forth above e.g., 5X SSC. In a preferred
10 embodiment, the T_m is 60°C ; in a more preferred embodiment, the T_m is 65°C .

A DNA "coding sequence" is a double-stranded DNA sequence which is transcribed and translated into a polypeptide in a cell *in vitro* or *in vivo* when placed under the control of appropriate regulatory sequences. The boundaries of the coding sequence are determined by a start codon at the 5' (amino) terminus and a translation stop
15 codon at the 3' (carboxyl) terminus. A coding sequence can include, but is not limited to, prokaryotic sequences, cDNA from eukaryotic mRNA, genomic DNA sequences from eukaryotic (e.g., mammalian) DNA, and even synthetic DNA sequences. If the coding sequence is intended for expression in a eukaryotic cell, a polyadenylation signal and transcription termination sequence will usually be located
20 3' to the coding sequence.

"Transcriptional and translational control sequences" are DNA regulatory sequences, such as promoters, enhancers, terminators, and the like, that provide for the expression of a coding sequence in a host cell. In eukaryotic cells, polyadenylation signals are control sequences.

25 A "promoter sequence" is a DNA regulatory region capable of binding RNA polymerase and initiating transcription of a downstream (3' direction) coding

sequence. For purposes of defining the present invention, the promoter sequence is bounded at its 3' terminus by the transcription initiation site and extends upstream (5' direction) to include the minimum number of bases or elements necessary to initiate transcription at levels detectable above background. Within the promoter sequence
5 will be found a transcription initiation site (conveniently defined for example, by mapping with nuclease S1), as well as protein binding domains (consensus sequences) responsible for the binding of RNA polymerase.

A "signal sequence" is included at the beginning of the coding sequence of a protein to direct the protein to a particular site/compartiment in the cell such as the surface of
10 a cell. This sequence encodes a signal peptide, N-terminal to the mature polypeptide, that directs the host cell to translocate the polypeptide. The term "translocation signal sequence" is used herein to refer to this sort of signal sequence. Translocation signal sequences can be found associated with a variety of proteins native to eukaryotes and prokaryotes, and are often functional in both types of organisms.

15 Identification of Genetic Mutations

A biological sample can be obtained from an individual and/or a blood relative of the individual, and from appropriate controls, using a sample from any body component including tissue punches, body fluids, and hair, as long as the biological sample contains nucleic acids and/or proteins/peptides. Thus the DNA, mRNA, proteins or
20 peptides of the biological sample can be used to identify mutations and/or variants in genes involved in folate, pyridoxine, or cobalamine metabolism. The present invention therefore includes methods of detecting and quantifying these nucleic acids and/or proteins/peptides that can be used to identify genetic risk factors.

In a particular embodiment the DNA is extractable. A particularly useful source of
25 DNA is blood. For example, 2.5- 40 mls of blood can be collected in a vacutainer

containing EDTA. The blood sample is placed on ice and then centrifuged to separate plasma, red cells, and buffy coat. The separated fractions are then frozen at -80°C.

The DNA can be isolated from the buffy coat by a number of procedures well known in the art including using a QIAmp column DNA extraction procedure or the

- 5 QIAGEN Genomic-tip method. The isolated DNA can be digested with a series of restriction enzymes, for example, and then the digested products can be hybridized with one or more particular nucleic acid probes designed from a particular gene to identify the gene and preferably to test for particular genetic mutations.

- Preferably the genomic DNA can be amplified by PCR using appropriate primer pairs
10 such as the primer pairs for the MTHFR or DHFR genes which were used in the Example below. The PCR amplified product can be sequenced directly, or alternatively be digested with one or more appropriate restriction enzymes. The resulting digested products can be separated *e.g.*, by column chromatography, or preferably by polyacrylamide or agarose gel electrophoresis. The isolated digestion
15 products can be compared *e.g.*, by previously determined restriction maps, and/or alternatively, the digestion products can be sequenced directly. Alternatively, as in the case of DHFR, genetic polymorphisms can be detected through the use of restriction enzymes.

- Although a restriction map of a gene is sufficient for the employment of the methods
20 disclosed herein, in preferred embodiments the nucleotide sequences of the genes used in the testing steps are known. To this end a large sampling of such sequences are provided in Tables 2-7. (These sequences may also be used in the design of restriction maps.) Thus, initially each gene whether used separately or used in a constellation of genes is characterized by the sequencing of the wild type gene,
25 preferably including the coding regions, introns, control sequences, and other non-coding regions. In addition, mutations of such genes found in the general population can also be characterized. With the recent advances in the sequencing of the human

genome the present invention contemplates that additional sequence information will become publicly available, particularly with regard to mutations in relevant introns, and control sequences etc. which are not available in cDNA libraries. Such sequence information is fully envisioned to be incorporated into the on-going compilations of relevant DNA sequence databases of the present invention, as well as for its parallel use in the general methodology described herein. Thus DNA or mRNA or cDNA made from the mRNA can be used to identify mutations and/or variants in genes involved in folate, pyridoxine, or cobalamine metabolism.

There are many methods currently known in the art to identify variant/mutant DNA, all of which may be used in the present invention (*see e.g.*, internet address <http://www.ich.bpmf.ac.uk/cmgs/mutdet.htm>). Such methods include but in no way are limited to direct sequencing, array sequencing, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (Malditof) [Fitzgerald *et al.*, *Ann. Rev. Biophy. Biomol. Struct.* **24**:117-140 (1995)], Polymerase Chain Reaction "PCR", reverse-transcriptase Polymerase Chain Reaction "RT-PCR", RNAase protection assays, Array quantitation *e.g.*, as commercially provided by Affymetrix, Ligase Chain Reaction or Ligase Amplification Reaction (LCR or LAR), Self-Sustained Synthetic Reaction (3SR/NASBA), Restriction Fragment Length Polymorphism (RFLP), Cycling Probe Reaction (CPR), Single-Strand Conformation Polymorphism (SSCP), heteroduplex analysis, hybridization mismatch using nucleases (*e.g.*, cleavase), Southern, Northern, Westerns, South Westerns, ASOs, Molecular beacons, footprinting, and Fluorescent *In Situ* Hybridization (FISH). Some of these methods are briefly described below.

PCR is a method for increasing the concentration of a segment of target sequence in a mixture of genomic DNA without cloning or purification. PCR can be used to directly increase the concentration of the target to an easily detectable level. This process for amplifying the target sequence involves introducing a molar excess of two oligonucleotide primers which are complementary to their respective strands of the

double-stranded target sequence to the DNA mixture containing the desired target sequence. The mixture is denatured and then allowed to hybridize. Following hybridization, the primers are extended with polymerase so as to form complementary strands. The steps of denaturation, hybridization, and polymerase extension can be
5 repeated in order to obtain relatively high concentrations of a segment of the desired target sequence. The length of the segment of the desired target sequence is determined by the relative positions of the primers with respect to each other, and, therefore, this length is a controllable parameter. Because the desired segments of the target sequence become the dominant sequences (in terms of concentration) in the
10 mixture, they are said to be "PCR-amplified." [Mullis (U.S. Patent No. 4,683,195) and Mullis et al. (U.S. Patent No. 4,683,202)]

In Ligase Chain Reaction or Ligase Amplification Reaction (LCR or LAR) four oligonucleotides, two adjacent oligonucleotides which uniquely hybridize to one strand of target DNA, and a complementary set of adjacent oligonucleotides, which
15 hybridize to the opposite strand are mixed and DNA ligase is added to the mixture. Provided that there is complete complementarity at the junction, ligase will covalently link each set of hybridized molecules. Importantly, in LCR, two probes are ligated together only when they base-pair with sequences in the target sample, without gaps or mismatches. Repeated cycles of denaturation, hybridization and ligation amplify a
20 short segment of DNA. [Barany, *Proc. Natl. Acad. Sci.*, **88**:189 (1991); Barany, *PCR Methods and Applic.*, 1:5 (1991); and Wu and Wallace, *Genomics* 4:560 (1989)] LCR has also been used in combination with PCR to achieve enhanced detection of single-base changes. Segev, PCT Public. No. W09001069 A1 (1990).

Self-Sustained Synthetic Reaction (3SR/NASBA) is a transcription-based *in vitro*
25 amplification system [Guatelli *et al.*, *Proc. Natl. Acad. Sci.*, **87**:1874-1878, 7797 (1990); Kwok *et al.*, *Proc. Natl. Acad. Sci.*, **86**:1173-1177) that can exponentially amplify RNA sequences at a uniform temperature. The amplified RNA can then be utilized for mutation detection (Fahy *et al.*, *PCR Meth. Appl.*, 1:25-33 (1991). In this

method, an oligonucleotide primer is used to add a phage RNA polymerase promoter to the 5' end of the sequence of interest. In a cocktail of enzymes and substrates that includes a second primer, reverse transcriptase, RNase H, RNA polymerase and ribo- and deoxyribonucleoside triphosphates, the target sequence undergoes repeated rounds of transcription, cDNA synthesis and second-strand synthesis to amplify the area of interest.

RFLP can be used to detect DNA polymorphisms arising from DNA sequence variation. This method consists of digesting DNA with one or more restriction endonucleases (*e.g.*, EcoRI) and analyzing the resulting fragments by means of Southern blots [Southern, E., *Methods in Enzymology*, **69**:152 (1980)], as further described by Botstein, et al., *Am. J. Hum. Genet.*, **32**:314-331 (1980) and White, et al., *Sci. Am.*, **258**:40-48 (1988). Since a DNA polymorphism may create or delete a restriction site, the length of the corresponding restriction fragment with any given restriction enzyme could change. Once a difference in a restriction fragment length is identified it can be used to readily distinguish a particular polymorphism from the wild type DNA. Mutations that affect the recognition sequence of the endonuclease will preclude enzymatic cleavage at that site, thereby altering the cleavage pattern of that DNA. DNAs are compared by looking for differences in restriction fragment lengths. A technique for detecting specific mutations in any segment of DNA is described in Wallace, *et al.*, [*Nucl. Acids Res.*, **9**:879-894 (1981)]. It involves hybridizing the DNA to be analyzed (target DNA) with a complementary, labeled oligonucleotide probe. Due to the thermal instability of DNA duplexes containing even a single base pair mismatch, differential melting temperature can be used to distinguish target DNAs that are perfectly complementary to the probe from target DNAs that differ by as little as a single nucleotide. In a related technique, described in Landegren, *et al.*, *Science*, **41**:1077-1080 (1988), oligonucleotide probes are constructed in pairs such that their junction corresponds to the site on the DNA being analyzed for mutation. These oligonucleotides are then hybridized to the DNA being analyzed. Base pair mismatch between either oligonucleotide and the target DNA at

the junction location prevents the efficient joining of the two oligonucleotide probes by DNA ligase.

- When a sufficient amount of a nucleic acid to be detected is available, there are advantages to detecting that sequence directly, instead of making more copies of that target, (*e.g.*, as in PCR and LCR). Most notably, a method that does not amplify the signal exponentially is more amenable to quantitative analysis. Even if the signal is enhanced by attaching multiple dyes to a single oligonucleotide, the correlation between the final signal intensity and amount of target is direct. Such a system has an additional advantage that the products of the reaction will not themselves promote further reaction, so contamination of lab surfaces by the products is not as much of a concern. Traditional methods of direct detection including Northern and Southern blotting and RNase protection assays usually require the use of radioactivity and are not amenable to automation. Recently devised techniques have sought to eliminate the use of radioactivity and/or improve the sensitivity in automatable formats.
- 15 One such example is the Cycling Probe Reaction (CPR) [Duck *et al.*, BioTech., 9:142 (1990)]. CPR uses a long-chimeric oligonucleotide in which a central portion is made of RNA while the two termini are made of DNA. Hybridization of the probe to a target DNA and exposure to a thermostable RNase H causes the RNA portion to be digested. This destabilizes the remaining DNA portions of the duplex, releasing the remainder of the probe from the target DNA and allowing another probe molecule to repeat the process. The signal, in the form of cleaved probe molecules, accumulates at a linear rate. While the repeating process increases the signal, the RNA portion of the oligonucleotide is vulnerable to RNases that may be carried through sample preparation.
- 25 Single-Strand Conformation Polymorphism (SSCP) is based on the observation that single strands of nucleic acid can take on characteristic conformations in non-denaturing conditions, and these conformations influence electrophoretic

mobility. [Hayashi, *PCR Meth. Appl.*, 1:34-38, (1991). The complementary strands assume sufficiently different structures that one strand may be resolved from the other. Changes in sequences within the fragment will also change the conformation, consequently altering the mobility and allowing this to be used as an assay for

5 sequence variations (Orita, *et al.*, *Genomics* 5:874-879, (1989). The SSCP process involves denaturing a DNA segment (*e.g.*, a PCR product) that is labeled on both strands, followed by slow electrophoretic separation on a non-denaturing polyacrylamide gel, so that intra-molecular interactions can form and not be disturbed during the run. This technique is extremely sensitive to variations in gel composition

10 and temperature.

In Fluorescent In Situ Hybridization (FISH), specific probes are designed which can readily distinguish the wild-type gene from the variant/mutant gene. Such methodology allows the identification of a variant/mutant gene through *in situ* hybridization (U.S. Patent No. 5,028,525, Issued July 2, 1991; U.S. Patent No.

15 5,225,326, Issued July 6, 1993; and U.S. Patent No. 5,501,952, Issued March 26, 1996. FISH does not require the extraction of DNA. In addition, procedures for separating fetal blood cells from maternal blood cells are well known in the art allowing the fetus and the mother to be analyzed from the same body fluid sample (*see* U.S. Patent No: 5,629,147, Issued May 13, 1997).

20 Similarly, antibodies raised against specific mutations and/or variants in the gene products of the genes involved in folate, pyridoxine, or cobalamine metabolism can be used to identify specific polymorphisms. Alternatively, antibodies raised against the wild type proteins can be used to detect and/or quantify the amount of wild type protein present in a given biological sample. In the case in which cross-reacting

25 protein isn't synthesized by the cells of an individual, or is synthesized in significantly lower amounts than those of control subjects, such determinations can be used to identify a genetic risk factor. In addition, these antibodies can be used in methods well known in the art relating to the localization and activity of the gene

products, *e.g.*, for Western blotting, imaging the proteins *in situ*, measuring levels thereof in appropriate physiological samples, etc. using any of the detection techniques known in the art. Furthermore, such antibodies can be used in flow cytometry studies, in immunohistochemical staining, and in immunoprecipitation
5 which serves to aid the determination of the level of expression of a protein in the cell or tissue.

In the particular instance when the gene product is an enzyme, *e.g.*, dihydrofolate reductase, the enzymatic activity of a biological sample can be indicative of the presence of a genetic risk factor. In a particular embodiment, a decrease in an enzyme
10 activity that is associated with folate, pyridoxine, or cobalamine metabolism can be indicative of the presence of the genetic risk factor. Such assays can be performed on multiple samples such as on a microplate reader [Widemann *et al.*, Clin Chem. 45:223-228 (1999)].

MODEL 1

15 THE GENE-TERATOGEN MODEL FOR THE INHERITANCE PATTERN OF CERTAIN DEVELOPMENTAL DISORDERS

Introduction:

It has long been known, *e.g.* from extensive studies of exogenous teratogens in inbred mice [Finnell and Chernoff, *Gene-teratogen* interactions: an approach to
20 understanding the metabolic basis of birth defects, In Pharmacokinetics in Teratogenesis, Vol. II:97-109 *Experimental Aspects In Vivo and In Vitro*, CRC Press, Inc, Boca Ratan, Fl. (1987)], that teratogens may be influenced by genetic factors. It is less well known that the same gene defect may cause different clinical disorders depending upon whether the metabolic effect of the gene defect is exerted during
25 gestation *in utero* or during postnatal life. However, the consequences of gene-teratogen interactions in human pedigrees have not been extensively explored, especially the consequences for the use of linkage mapping to identify an unknown gene acting *in utero* to cause a developmental disorder. A number of common human

- developmental disorders have been shown to have a genetic component to their etiology. However, for certain developmental disorders, the mode of inheritance has been difficult to determine and linkage studies have met with unexpected difficulties or have achieved limited success. These developmental disorders include spina bifida cystica [Chatkupt, *Am J Med Genet*, 44:508-512 (1992)], Tourette's syndrome & related disorders, e.g. obsessive-compulsive disorder and chronic multiple tics syndrome [Pauls, *Adv Neurol*, 58:151-157 (1992); McMahon *et al.*, *Adv Neurol*, 58:159-165 (1992); Heutink *et al.*, *Am J Hum Genet*, 57:465-473 (1995); Grice *et al.*, *Am J Hum Genet*, 59:644-652 (1996)], learning disorders, including dyslexia [Lewis, *et al.*, *Behav Genet*, 23:291-297 (1993); Pennington, *J Child Neurol 10 Suppl*, 1:S69-S77 (1995)], conduct disorder [Lombroso *et al.*, *J Am Acad Child Adolesc Psychiatry*, 33:921-938 (1994)], attention-deficit hyperactivity disorder [Lombroso *et al.*, *J Am Acad Child Adolesc Psychiatry*, 33:921-938 (1994)], bipolar illness [Baron, *Acta Psychiatr Scand*, 92:81-86 (1995); Benjamin and Gershon, *Biol Psychiatry*, 40:313-316 (1996); Risch and Botstein, *Nature Genet*, 12:351-353 (1996); Jamison and McInnis, *Nature Med*, 2:521-522 (1996); Morell, *Science*, 272:31-32 (1996)], schizophrenia [Owen, *Psychol Med*, 22:289-293 (1992); Cloninger, *Am J Med Genet*, 54:83-92 (1994); Lander and Kruglyak, *Nature Genet*, 11:241-247 (1995); Baron, *Acta Psychiatr Scand*, 92:81-86 (1995); Benjamin and Gershon, *Biol Psychiatry*, 40:313-316 (1996); Baron, *Am J Med Genet*, 67:121-123 (1996)], autism [Lombroso *et al.*, *J Am Acad Child Adolesc Psychiatry*, 33:921-938 (1994)], and obsessive-compulsive disorder in adults [Lombroso *et al.*, *J Am Acad Child Adolesc Psychiatry*, 33:921-938 (1994)]. A recent article [Moldin, *Nature Genet*, 17:127-129 (1997)] has reviewed "The maddening hunt for madness genes."
- 25 The present model addresses the question of the mode of inheritance of certain developmental disorders and proposes the "gene-teratogen model." The model suggests that the mode of inheritance of genes acting prenatally may in some cases be fundamentally different from that of genes acting postnatally. Even the same gene acting prenatally may produce a different disorder from that gene acting postnatally.

The inheritance pattern in the gene-teratogen model is simple, but from the perspective of the patient with the developmental disorder is neither dominant nor recessive. Some disorders regarded as multifactorial, polygenic, or oligogenic may have this mode of inheritance. In the gene-teratogen model, genetically determined
5 teratogen production by the mother during pregnancy damages the fetus producing the abnormal phenotype of a developmental disorder. The model is illustrated with two types of loci, 1. a teratogenic locus acting in the mother, and 2. a modifying or specificity locus acting in the fetus. Damage by the teratogen is influenced also by environmental factors. The model is interesting because it is simple and because
10 teratogenic loci will be difficult to locate by parametric or non-parametric linkage mapping techniques due to misspecification of the affection status of both mother and affected children. A study design is suggested for identifying teratogenic loci. An example of the gene-teratogen model is the major intrauterine effect seen in offspring of phenylketonuric mothers. Certain developmental disorders whose mode of
15 inheritance has been difficult to determine or whose genetic factors have been difficult to locate are candidates for the gene-teratogen model, including spina bifida cystica, Tourette's syndrome, learning disorders including dyslexia, conduct disorder, attention-deficit hyperactivity disorder, bipolar illness, schizophrenia, autism, and obsessive-compulsive disorder.

20

The Gene Teratogen Model

The model is described in Table 1 using two kinds of loci: a "teratogenic" locus and a "modifying" or "specificity" locus. The gene-teratogen model requires a teratogenic locus. One or more modifying or specificity loci may or may not be present. Also, two types of phenotypes are defined: 1. the teratogen-induced phenotype; and 2. the
25 teratogenic phenotype, *i.e.*, the phenotype of a mother that produces a teratogenic effect during pregnancy. The two phenotypes are different for the teratogenic locus but are identical for the modifying or specificity loci.

TABLE 1
DIAGRAM OF THE GENE-TERATOGEN MODEL

5

Grandparents:	Maternal Grandmother AabbCCdd	Maternal Grandfather AaBbCcdd	Paternal Grandmother AAbbCcDd	Paternal Grandfather AAbbCCdd
Parents:	Mother aaBbCcdd		Father AAbbCcDd	
Child:	Child (fetus) with developmental disorder AabbccDd			
locus A:	teratogenic locus, recessive, acting in the mother to cause intrauterine teratogenic damage to the fetus.			
locus B:	teratogenic locus, dominant, acting in the mother to cause intrauterine teratogenic damage to the fetus.			
locus C:	modifying or specificity locus, recessive, acting in the fetus.			
locus D:	modifying or specificity locus, dominant, acting in the fetus.			

- 10 The teratogenic locus may be dominant (locus A) or recessive (locus B). This locus acts in the mother during pregnancy to cause an intrauterine teratogenic effect in the fetus. The teratogenic effect may result from the production of an endogenous teratogen, from potentiation of an exogenous teratogen, from a metabolic deprivation or imbalance or from some other mechanism. Only one teratogenic locus is required;
- 15 both locus A and locus B are shown on the same diagram for simplicity. A specificity or modifying locus may be dominant (locus C) or recessive (locus D). Such a locus acts during pregnancy or after to modify the extent of the developmental damage done by the teratogenic locus or even to prevent or repair the damage. For example, for a teratogen acting at a certain time in development, locus C or D may determine
- 20 whether brain or kidney is damaged, which structures of the brain are damaged, or whether damage occurs at all.

1. Locus A, recessive teratogenic locus, acting in the mother: The child is the patient with the abnormal phenotype of a specific developmental disorder, while mother,

father, and grandparents do not have the abnormal phenotype of that disorder (Table 1). Locus A acts in the mother during pregnancy causing her to produce the teratogenic effect that damages the developing fetus leading to the developmental disorder either in the fetus or postnatally in the child or adult. Since this locus is recessive in action, the mother, a homozygote (aa) for the disease allele, is the genetic "patient." Her abnormal phenotype, the "teratogenic phenotype", is the trait of producing the teratogenic effect during pregnancy. Her fetus, damaged by the teratogenic effect *in utero*, does develop the teratogen-induced phenotype. However, the fetus is only a heterozygote (Aa) at locus A and thus lacks both the abnormal homozygous genotype at locus A and the abnormal teratogenic phenotype; *e.g.*, if the fetus is a daughter, she will not produce the teratogenic effect later during pregnancy. Thus, the fetus is affected with the developmental disorder but is not the genetic "patient." Locus A, acting through a teratogenic effect, cannot be the only etiological factor for the developmental disorder. If it were, then all pregnancies of an aa mother would have the teratogen-induced phenotype which is not the case. Environmental and/or other genetic factors, are required. An aa father will have the abnormal genotype, but not the abnormal teratogenic phenotype because he could never become pregnant.

2. *Locus B, dominant teratogenic locus acting in the mother:* The situation is the same as for locus A except that locus B is dominant in action (Table 1). The mother has the abnormal genotype, Bb, and the abnormal teratogenic phenotype. The fetus has the teratogen-induced phenotype but in the instance shown (Table 1) has neither the abnormal genotype, the teratogenic phenotype, nor even a copy of the disease allele. The maternal grandfather shown (Table 1) has the abnormal genotype, Bb, but does not have the teratogenic phenotype because he could never become pregnant.

3. *Environmental effects:* The teratogenic effect is modified by environmental factors, *e.g.* maternal dietary factors, infection, or ingestion of teratogen. These environmental factors may interact with locus A or B or may act independently. From

the perspective of the fetus later to develop the developmental disorder (teratogen-induced phenotype), intrauterine teratogenic is an environmental not a genetic effect.

4. *Modifying or Specificity Loci Acting in the Fetus, Loci C & D*: These loci may
- 5 interact with the teratogenic locus or the environmental factors to increase or decrease their effect, or alternatively could act independently. Such genetic factors may be recessive (locus C) or dominant (locus D). Genotypes and phenotypes of locus C and D behave conventionally with respect to the developmental disorder. For locus C and D, the fetus is with the developmental disorder is now the genetic "patient". Maternal
- 10 teratogenic *in utero* is an environmental effect. It is thus possible that the same gene locus could act in part as a teratogenic locus and in part as a modifying or specificity locus.

DISCUSSION

- The Example of Phenylketonuria*: An example of the gene-teratogen model is the
- 15 major intrauterine effect in maternal phenylketonuria (PKU). Phenylketonuria itself is a recessive postnatal disorder. Untreated homozygous PKU mothers and fathers both have elevated blood phenylalanine (hyperphenylalaninemia). However, heterozygous offspring of untreated PKU mothers (but not fathers) have an abnormal phenotype.[Koch *et al.*, *Acta Paediatr Suppl*, 407:111-119 (1994); Allen *et al.*, *Acta*
- 20 *Paediatr Suppl*, 407:83-85 (1994); Abadie *et al.*, *Archives Pediatr*, 3:489-486 (1996)]. Thus the elevated blood phenylalanine or other metabolite(s) in the mother acts as a teratogen for the fetus. Note that the fetus of an untreated phenylketonuric mother does not have the phenotype of PKU (the "teratogenic phenotype"), but has a different phenotype (the "teratogen-induced phenotype").
- 25 Phenylketonurics [Menkes, *Textbook of Child Neurology*, Lea & Febiger, Philadelphia (1990)] are normal at birth and develop a progressive disorder postnatally characterized by vomiting, eczema, seizures (infantile spasms with hypsarrhythmia on electroencephalography), and mental retardation. The fetus of an

untreated phenylketonuric mother [Menkes, *Textbook of Child Neurology*, Lea & Febiger, Philadelphia (1990)] has a congenital non-progressive disorder of fetal origin characterized by microcephaly, abnormal facies, mental retardation, congenital heart disease, and prenatal and postnatal growth retardation. The PKU phenotype is a postnatal degenerative disorder; the phenotype of the PKU intrauterine effect is a developmental disorder. The teratogenic effect is not dependent upon the fetal genotype, although the fetus is an obligate heterozygote since the mother is a homozygote for phenylketonuria and the father (usually) has the normal genotype. Thus, in phenylketonuria, a mutation at the same gene locus causes two distinct disorders depending upon whether the period of abnormal gene action is prenatal or postnatal. A fetus with the abnormal homozygous genotype who is carried by a heterozygous mother is protected *in utero*, but develops PKU postnatally. A heterozygous fetus carried by a mother with the abnormal homozygous genotype is damaged *in utero* when the mother's genotype predominates, but is protected from PKU postnatally by its own genotype.

An Example from Studies in Inbred Mice: Finnell and Chernoff [*Gene-teratogen interactions: an approach to understanding the metabolic basis of birth defects, In Pharmacokinetics in Teratogenesis, Vol. II:97-109 Experimental Aspects In Vivo and In Vitro*, CRC Press, Inc, Boca Ratan, Fl. (1987)] have reviewed a group of elegant experiments in inbred mice documenting that differences in susceptibility to exogenous teratogens can be regarded as a genetic trait that is determined by susceptibility or liability genes of either the maternal or fetal genotype [Finnell and Chernoff, *Gene-teratogen interactions: an approach to understanding the metabolic basis of birth defects, In Pharmacokinetics in Teratogenesis, Vol. II:97-109 Experimental Aspects In Vivo and In Vitro*, CRC Press, Inc, Boca Ratan, Fl. (1987)]; Finnell *et al.*, *Am J. Med. Genet.* **70**:303-311 (1997); Bennett *et al.*, *Epilepsia* **38**:415-423 (1997)]. For example, sensitivity to acetazolamine-induced ectrodactyly is determined by the presence of three genes, and the fetus must be homozygous for the recessive allele at all three loci in order to express the malformation. However, the

inbred mouse models used do not mirror the human situation in at least three respects. First, the human population is an outbred population compared to these inbred mouse models. Consequently, the relevant genotypes may be highly variable among members of different families. Second, the inbred mouse experiments address the
5 question of exogenous rather than endogenous teratogens. Third, the inbred mouse studies rely upon known or candidate susceptibility loci, whereas in humans, the problem has been to locate and identify disease unknown loci largely by using linkage mapping techniques.

Implications for Linkage Mapping:

- 10 *Teratogenic Locus (Locus A or B):* The gene-teratogen model has major implications for linkage mapping done with either parametric or non-parametric methods. The problem for both methods is incorrect assignment of affection status. In the lod score method, a genetic model of the disease is constructed and an affection status is assigned to each member of the pedigree. If the genetic model specified is wrong, the
15 linkage results may be falsely positive or falsely negative [Terwilliger and Ott, *Handbook of Human Genetic Linkage*, Johns Hopkins Univ. Pr., Baltimore (1994)].

In developmental disorders resulting from the gene-teratogen model, the phenotype assignment for lod score analysis will be incorrect. The patient with the developmental disorder will be assigned the affected phenotype, whereas the patient
20 is actually affected only for the teratogen-induced phenotype, but is unaffected for the teratogenic phenotype. Likewise, the mother will be assigned the unaffected phenotype for linkage analysis. Actually, she is unaffected only for the teratogen-induced phenotype, but is affected for the teratogenic phenotype. Lod scores should increase when phenotype assignments have been corrected. However,
25 apparently dominant inheritance may in fact turn out to be pseudodominant if the mutant allele is common in the population. For non-parametric analysis, a similar misassignment occurs. In the case of affected sib-pairs, the affected sibs will be assigned the affected phenotype. Actually, the sibs are affected only for the

teratogen-induced phenotype, but are unaffected for the teratogenic phenotype. The mother will be assigned the unaffected or unknown phenotype. Actually, she is unaffected only for the teratogen-induced phenotype but is affected for the teratogenic phenotype. Thus, the "affected sib-pair" families are likely to turn out to contain only
5 a single sporadic case, since the only individual in the kindred affected with the teratogenic phenotype will be the mother.

For the transmission/disequilibrium test (TDT) [Spielman *et al.*, *Am J Hum Genet*, 52:506-516 (1993); Ewens and Spielman, *Am J Hum Genet*, 57:455-464 (1995)] the patient with the developmental disorder will be assigned the affected phenotype.
10 Actually, the patient will be affected only for the teratogen-induced phenotype but will be unaffected for the teratogenic phenotype. The mother will be assigned the unaffected or unknown phenotype. Actually, she is unaffected only for the teratogen-induced phenotype but is affected for the teratogenic phenotype. The expectation of TDT is that alleles of a linked locus will show distortion from random
15 transmission from mother (or father) to the patient. Since the patient is unaffected for the teratogenic phenotype, no transmission distortion from mother (or father) to child will be observed. Transmission distortion for alleles of a teratogenic locus will in fact occur from the mother's parents to the mother, the actual patient for the teratogenic phenotype. But this will not be looked for because the phenotypes have been wrongly
20 assigned. In addition, grandparents of the patients with the developmental disorder have probably not had DNA collected. Therefore, for the TDT, negative results may occur for disease alleles of a teratogenic locus because incorrect phenotype assignments will have been made. When correct phenotype assignments have been made, transmission distortion to the mother from her parents should be expected for
25 disease alleles of a teratogenic locus. Analogous misassignments are made in allelic association and haplotype relative-risk analyses [Falk and Rubinstein, *Ann Hu, Genet*, 51:227-233 (1987); Terwilliger and Ott, *Hum Hered*, 42:337-346 (1992); Thomson, *Am J Hum Genet*, 57:487-498 (1995)].

Modifying or Specificity Loci (Locus C and/or D): Since these loci behave in a conventional fashion, the phenotype assignments will be correct. Consequently, genes identified by conventional parametric or non-parametric linkage studies are likely to be modifying or specificity loci. An important question for linkage mapping

5 is the relative contribution to the abnormal phenotype of the developmental disorder made by the teratogenic locus versus that of a modifying or specificity locus. If the effect of a teratogenic locus is small, then loci identified by conventional linkage studies will be specificity or modifying loci and the mode of inheritance will be Mendelian or multifactorial. If a teratogenic locus makes a major contribution to

10 phenotype, then linkage mapping studies will not give a consistent answer and the mode of inheritance will be difficult to determine.

The presence of a teratogenic locus may be suspected if the maternal contribution to phenotype is different from or greater than the paternal contribution. For example, the mother's relatives of spina bifida infants more frequently have affected children

15 than the father's relatives. Suggested explanations for this observation have been mitochondrial inheritance, maternal effect, or genomic imprinting [Chatkupt, *Am J Med-Genet*, 44:508-512 (1992)]. The operation of a teratogenic locus is another explanation and is itself a form of maternal effect. For a recessive teratogenic locus, the mother's sisters would be at greatest risk of having offspring with the

20 teratogen-induced phenotype.

Implications for Definition of Phenotype: All the pregnancies of a mother with the teratogenic phenotype are at risk for the developmental disorder, the teratogen-induced phenotype. Yet only a few of the fetuses will be affected by the developmental disorder because of the action of environmental factors and/or the

25 modifying or specificity loci. The action of the environmental factors is fully quantitative: depending upon the amplitude of the environmental effect, a mild, moderate, or severe teratogen-induced phenotype may result. In addition, the environmental factor may act at different times in fetal development producing

qualitatively different phenotypes. Thus, quantitatively or qualitatively different teratogen-induced phenotypes may result from pregnancies of the same mother with the teratogenic phenotype. In addition, the action of the modifying or specificity loci may produce quantitatively or qualitatively different phenotypes in offspring of the same couple. Such different phenotypes may be diagnostically classified as different disorders. This may complicate attempts at associating specific loci with a specific teratogen-induced phenotype. All of the teratogen-induced phenotypes resulting from pregnancies of a mother with the teratogenic phenotype modified only by environmental factors are genetically indistinguishable. However, such teratogen-induced phenotypes affected also by the various modifying or specificity loci segregating among the offspring of a single couple are only partially genetically related.

Methods to Identify Teratogenic Loci: One effective approach to finding a putative teratogenic locus is to carry out non-parametric linkage studies of families consisting of a patient affected with the developmental disorder, the patient's two (unaffected) parents, and the patient's four (unaffected) grandparents (Table 1). In such a family, the mother is the genetic patient but the other family members are not. Now, the mother's nuclear family (the mother and her parents) is compared with the father's nuclear family (the father and his parents). In a haplotype relative risk study, the disease allele(s) of the teratogenic locus will occur more frequently in the mother compared with other alleles of her parents; the disease allele(s) of the teratogenic locus will not occur more frequently in the father compared with other alleles of his parents. In a transmission/disequilibrium test, transmission distortion will be seen for the disease allele(s) of a teratogenic locus in the mother's nuclear family but not in the father's nuclear family. In an allelic association study, the disease allele will occur more frequently in mothers, patients (with the developmental disorder), and patient's sibs (both affected and unaffected) than in unrelated control individuals. Disease allele frequency in fathers will not be distinguishable from that in control individuals.

Certain developmental disorders with a genetic component to etiology, whose mode of inheritance has been difficult to determine or whose genetic factors have been difficult to locate, including those mentioned earlier, are candidates for the gene-teratogen model.

5

MODEL 2:

DNA POLYMORPHISM-DIET-COFACTOR-DEVELOPMENT HYPOTHESIS
FOR SCHIZOPHRENIA AND OTHER DEVELOPMENTAL DISORDERS

Folate metabolism is complex. At least 30 gene loci are involved in absorption, transport, and metabolism of folate, and these are regulated by additional gene loci.

10 Any of these is potentially a genetic risk factor for schizophrenia, although MTHFR and DHFR are particularly good candidates. Likewise, genes encoding proteins involved in the pathways of other vitamin-cofactors may be genetic risk factors.

Two cofactors that may be of particular potential importance are cobalamin and pyridoxine. Cobalamin is relevant because its metabolism is closely intertwined with

15 that of folate. For example, cobalamin is required for the activity of methionine synthase (MTR), a folate-related enzyme. Decreased cobalamin can affect folate metabolism through the folate trap. Pyridoxine is relevant because the pyridoxine-dependent enzyme cystathionine beta-synthase (CBS), along with the cobalamin-dependent enzyme MTR and folate pathways including MTHFR and

20 DHFR all participate in catabolism of homocysteine, an amino acid that is suspected of being a teratogen during pregnancy. Also, kynureninase, an important enzyme affecting niacin metabolism and serotonin synthesis is pyridoxine-dependent. Therefore, mutations of the genes encoding such proteins, especially common polymorphisms, could play a role in the cause of schizophrenia.

25 Since folate, cobalamin, and pyridoxine are all dietary constituents, the dietary content of these cofactors could be lead to an "environmental" generation of a risk

factor for schizophrenia. In addition genes encoding proteins involved in folate, cobalamin, and pyridoxine metabolism and catabolism could be genetic risk factors for schizophrenia. Thus, the cofactors and the proteins involved in pathways relevant to these cofactors can potentially have either or both environmental and genetic effects on the susceptibility of an individual on schizophrenia.

Since the genetic aspect of schizophrenia differs so profoundly from other disorders which have been identified by linkage mapping techniques, it is clear that a new model for the genetic connection to schizophrenia is required. Therefore, the DNA Polymorphism-Diet-Cofactor-Development (DDCD) hypothesis, is disclosed herein.

10 The DDCD hypothesis is that interacting genetic and environmental factors affecting the metabolism of folate, cobalamin, or pyridoxine or all of these, play a role in the etiology of schizophrenia. The genetic effect results from the aggregate effect of multiple mutations that individually, for the most part, have small effects on folate-, cobalamin- or pyridoxine-related genes, some of which will be common in the population, and can act *in utero*. Environmental factors include dietary folate and cobalamin and pyridoxine. If schizophrenia results from mild deficiency during fetal development of dietary folate, cobalamin, or pyridoxine potentiated by mild genetic susceptibility mutations of genes related to these cofactors and by pregnancy, then this would be difficult to document by linkage mapping techniques. An example of interaction of genetic and environmental factors is that genetic factors are important for incorporating dietary folate; the enzyme dihydrofolate reductase is required for conversion of dietary folate to folinic acid thus allowing dietary folate to enter the body's metabolic pathways. Another example is that folate and cobalamin requirements increase during pregnancy; thus pregnancy could potentiate the effects of mild genetic defects of mother, fetus, or both. Deficiencies of a vitamin are often part of a broader dietary deficiency affecting multiple nutrients in addition to the vitamin being measured.

Locus Heterogeneity: The metabolic pathways of folate, cobalamin, and pyridoxine are complex and related to each other. Multiple gene loci code for the enzymes and transport proteins are required (Tables 2-7). Thus, a defect of folate, cobalamin, or pyridoxine metabolism could result from the aggregate effect of multiple mutations
5 each of relatively small effect interacting with environmental factors. Different individuals might have different combinations of mutations. Such a metabolic defect would be difficult to detect by linkage mapping techniques because of locus heterogeneity.

Alternatively, even if one genetic defect were sufficient to make an individual more
10 susceptible to having schizophrenic offspring, for example, because of the large number of potential genetic factors, and the corresponding importance of environmental factors, elucidation of such an individual genetic defect would still be difficult unless, of course, the genetic defect caused a major effect. The difficulty in elucidating an individual genetic defect is magnified when the genetic factor acts in
15 the mother, and not in the schizophrenic patient.

High Disease Allele Frequency: Numerous mutational variants of folate and cobalamin genes are known. Some of these have functional significance and in addition are sufficiently common in a given population to be regarded as genetic polymorphisms. However, these common alleles are unlikely to have a major
20 harmful effect by themselves, for if they did they would become uncommon in the population in the absence of selection effects, and would likely appear as Mendelian disorders. Thus, the folate, cobalamin, or pyridoxine disease alleles related to schizophrenia would appear to be more likely those of minor deleterious effect or those with harmful effect only in the presence of environmental deficiencies or
25 pregnancy. Such disease genes of high population frequency will be difficult to detect by linkage mapping methods because high disease allele frequency decreases the power of linkage studies [Terwilliger and Ott, *Handbook of Human Genetic Linkage*, John Hopkins Univ. Press, Baltimore, (1994)].

- Developmental Genes:* Folate, cobalamin, and pyridoxine defects act prenatally as well as postnatally. Folate, cobalamin, and pyridoxine metabolism are crucial for DNA synthesis and cell division, which are of disproportionate importance during brain development. Some defects of folate, cobalamin, or pyridoxine metabolism
- 5 elevate blood homocysteine, a toxic and potentially teratogenic substance. Genes acting in the mother to damage the developing fetus, *e.g. via* the gene-teratogen model (Model 1, above), have a mode of inheritance that is neither dominant nor recessive with respect to the fetus. Attempts to assign a mode of inheritance in this situation will be unsatisfactory because affection status would be incorrectly assigned.
- 10 The mode of inheritance of a developmental disorder resulting from a teratogenic locus would be regarded as either multifactorial or unknown. This is the situation with schizophrenia whose mode of inheritance is unknown. Use of an incorrect genetic model decreases the power of a linkage studies [Terwilliger and Ott, *Handbook of Human Genetic Linkage*, John Hopkins Univ. Press, Baltimore,
- 15 (1994)].

- Genes of Folate Metabolism:* Folate metabolism is extremely complex [Rosenblatt, In: *The Metabolic and Molecular Bases of Inherited Disease*, Scriver *et al.* (eds), New York: McGraw-Hill, pp. 3111-3128 (1995); Mudd *et al.*, In: *The Metabolic and Molecular Bases of Inherited Disease*, Scriver *et al.* (eds), New York: McGraw-Hill
- 20 pp. 1279-1327 (1995)]. At least 30 gene loci (Table 2) have been identified as folate-related. These contribute to folate mediated 1-carbon transfer reactions, binding, transport and metabolism of folate, and other functions. A number of these have been cloned and localized to a chromosomal region (Table 3).

TABLE 2

FOLATE-RELATED GENES/ENZYMES/TRANSPORTERS*

	Folate-Related Genes/Enzymes/Transporters*	SEQ ID NO:
	methylenetetrahydrofolate reductase, MTHFR, MIM 236250	1
5	methionine synthase (methyltetrahydrofolate:L-homocysteine S-methyltransferase), MTR, MIM 156570	2
	dihydrofolate reductase, DHFR, MIM 126060	3
	folylpolyglutamate synthase, FPGS, MIM 136510	4
10	folate receptor 1, folate receptor alpha (FOLR1, adult; FR-alpha), MIM 136430	5
	folate receptor 2, folate receptor beta (FOLR2, fetal; FR-beta), MIM 136425 (a.a.)	6
	folate receptor 2-like (FOLR2L, fetal-like), MIM-none	
	folate receptor gamma (FR-gamma), MIM 602469	7
15	serine hydroxymethyltransferase 1, SHMT1, MIM 182144	8
	methylenetetrahydrofolate dehydrogenase, methenyltetrahydrofolate cyclohydrolase, 10-formyltetrahydrofolate synthetase (trifunctional enzyme, MTHFD), MIM 172460	9
	serine hydroxymethyltransferase 2, SHMT2, MIM 138450	10
20	thymidylate synthase, TYMS, MIM 188350	11
	GAR (5-phosphoribosylglycineamide) transformylase, GART, MIM 138440	12
	reduced folate carrier-1, RFC1. Probably identical to micromolar membrane transport protein, intestinal folate carrier-1 (IFC1), and neutral folate transport protein. MIM 600424	13
25	cystathionine beta-synthase, CBS, MIM 236200	14
	AICAR (5-phosphoribosyl-5-aminoimidazole-4-carboxamide) transformylase	15
	glutamate formiminotransferase, MIM 229100	
	forminotetrahydrofolate cyclodeaminase	
	5, 10-methenyltetrahydrofolate synthetase	16
30	10-formyltetrahydrofolate dehydrogenase, Mim 600249	

Folate-Related Genes/Enzymes/Transporters*		SEQ ID NO:
5	glycine cleavage pathway (SHMT plus three enzymes): MIM 238331 Gly-decarboxylase MIM 238300 H-Protein MIM 238330 T-Protein MIM 238310	17 18 19
	cblG (affects function of MTR), MIM 250940	
	methionine adenosyltransferase 1, MAT1A, (ATP:L-methionine S-adenosyltransferase), MIM 250850	20
	pteroyl polyglutamate hydrolase ("conjugase"), form 1	
10	pteroyl polyglutamate hydrolase ("conjugase"), form 2	
	NAD-dependent enzyme methylene tetrahydrofolate dehydrogenase cyclohydrolase (a.a.)	21
15	methionine adenosyltransferase 2, MAT2A, MIM 601468	22
	5-methyltetrahydrofolate- homocysteine methyltransferase reductase (MTRR) MIM 602568; #Variant in MTRR linked to cblE MIM 236270	23
	methyltransferases	
	S-adenosylmethionine decarboxylase, MIM 180980	24
20	decarboxylated S-adenosylmethionine:putrescine propylaminotransferase or spermidine synthetase (a.a.)	25
	S-adenosylhomocysteine hydrolase, , MIM 180960	26
	betaine-homocysteine methyltransferase dimethylthetin-homocysteine methyltransferase	27
	gamma-cystathionase (L-cystathionine cysteine-lyase (deaminating)), MIM 602888	28
25	folic acid transport protein, MIM 229050	
	DHFR (exon 6 and 3' flanking region)	30
	kynureninase	35
30	human DHFR, exons 1 and 2 [Chen <i>et al.</i> , <i>J. Biol. Chem.</i> 259:3933-3943 (1984)]	36
	*listed with alternate names, abbreviations, and MIM numbers; #cblE is a phenotype for a particular group of disorders of folate/cobalamin metabolism. (a.a.) indicates the amino acid sequence	

TABLE 3
LOCALIZED GENE LOCI RELATED TO FOLATE METABOLISM

	Gene/enzyme/transport protein	Location	References
	MTHFR	1p36.3	Goyette <i>et al.</i> , (1994); *, **
5	MTR	1q43	Cook and Hamerton, (1979); Mellman <i>et al.</i> , (1979) **
	DHFR	5q11.2-13.2	Weiffenbach <i>et al.</i> , (1991) Gilliam <i>et al.</i> (1989b) *, **
	FPGS	9cen-q34	Jones and Kao (1984); Walter <i>et al.</i> (1992) *, **
	MAT	10q22	**
	FR	11q13.3-q14.1 11q13.3-113.5	Lacey <i>et al.</i> (1989), Ragoussis <i>et al.</i> , (1992); Ratnum <i>et al.</i> (1989); Walter <i>et al.</i> (1992); * Ragoussis <i>et al.</i> , (1992), **
10	SHMT2	12q12-q14 12q13	Garrow <i>et al.</i> , (1993); Law and Kao, (1979) * **
	MTHFD	14q24	Rozen <i>et al.</i> , (1989), Jones <i>et al.</i> (1981), *, **
	LCCL	16pter-qter	*, **
	SHMT1	17p11.2	Garrow <i>et al.</i> , (1993) *, **
	TYMS	18p11.31.-p11.22 18p11.32	* Hori <i>et al.</i> , (1990); Silverman <i>et al.</i> , (1993)
15	SAHH	20cen-q13.1	*
	GART	21q22.1	McInnis <i>et al.</i> (1993) Schild <i>et al.</i> (1990) Avrarmopoulos <i>et al.</i> (1993) Goto <i>et al.</i> (1993) *, **
	RFC1	21q22.2-22.3	Moscow <i>et al.</i> , (1995)

Gene/enzyme/transport protein	Location	References
CBS	21q22.3	Munke <i>et al.</i> , (1988)
5	notes: MTHFR=methylenetetrahydrofolate reductase. MTS=methionine synthase. DHFR=dihydrofolate reductase. FPGS=folylpolyglutamate synthase. MAT=methionine adenosyltransferase, (ATP:L-methionine S-adenosyltransferase). FR=folate receptor complex: FR-alpha=FOLR1=folate receptor 1, adult; FR- beta=FOLR2=folate receptor 2, fetal; FR-gamma; FOLR2L=folate receptor 2-like. SHMT2=serine hydroxymethyltransferase 2, mitochondrial. MTHFD=5, 10- methylenetetrahydrofolate dehydrogenase, 5, 10-methylenetetrahydrofolate cyclohydrolase, 10-formyltetrahydrofolate synthase (trifunctional enzyme). 10 LCCL=gamma-cystathionase (L-cystathionine cysteine-lyase (deaminating). SHMT1=serine hydroxymethyltransferase 1, soluble. TYMS=thymidylate synthetase. SAHH, S-adenosylhomocysteine hydrolase. GART=phosphoribosylglycineamide formyltransferase. RFC1=reduced folate carrier-1 (possibly identical to IFC1, intestinal folate carrier-1). CBS=cystathionine 15 beta-synthase. Location information from GOD (*), from MIM (**). Goyette <i>et al.</i> , <i>Nat. Gen.</i> 7:195-200 (1994) Cook and Hamerton, <i>Cytogenet Cell Genet.</i> 25:9-20 (1979) Mellman <i>et al.</i> , <i>Proc. Natl. Acad. Sci.</i> 76:405-409 (1979) Weiffenbach <i>et al.</i> , <i>Genomics</i> 10:173-185 (1991) 20 Gilliam <i>et al.</i> , <i>Genomics</i> 5:940-944 (1989b) Jones and Kao, <i>Cytogenet Cell Genet.</i> 37: 499 (1984) Walter <i>et al.</i> , <i>Ann. Hum. Genet.</i> 56:212 (1992) Lacey <i>et al.</i> , <i>Am.J. Med. Genet.</i> 60:172-173 (1989) Ragoussis <i>et al.</i> , <i>Genomics</i> 14:423-430 (1992) 25 Ratnum <i>et al.</i> , <i>Biochem.</i> 28:8249-8254 (1989) Garrow <i>et al.</i> , <i>J. Biol. Chem.</i> 268:11910-11916 (1993). Law and Kao, <i>Cytogenet Cell Genet.</i> 24: 102-114 (1979) Rozen <i>et al.</i> , <i>Ann. Hum. Genet.</i> 44:781-786 (1989) Jones <i>et al.</i> , <i>Somat. Cell Genet.</i> 7:399-409 (1981) 30 Hori <i>et al.</i> , <i>Hum. Genet</i> 85:576-580 (1990) Silverman <i>et al.</i> , <i>Genomics</i> 15:442-445 (1993) McInnis <i>et al.</i> , <i>Genomics</i> 16:562-571 (1993) Schild <i>et al.</i> , <i>Proc. Natl. Acad. Sci</i> 87:2916-2920 (1990) Avramopoulos <i>et al.</i> , <i>Genomics</i> 15:98-102 (1993) 35 Goto <i>et al.</i> , <i>Neuromusc Disord.</i> 3:157-160 (1993) Moscow <i>et al.</i> , <i>Cancer Res.</i> 55:3790-3794 (1995) Munke <i>et al.</i> , <i>Am J. Hum. Gen.</i> 42:550-559 (1988)	

Genes of Cobalamin Metabolism: Cobalamin metabolism is also complex [Benton and Rosenberg, In: *The Metabolic and Molecular Bases of Inherited Disease*,

- 40 Disease, Scriver *et al.* (eds), New York: McGraw-Hill, 3129-3149 (1995)]. At least 15 gene loci (Table 4) have been identified as cobalamin-related. These contribute to

- the binding, transport, and metabolism of cobalamin, and its functions. A number of these have been cloned and localized to a chromosomal region (5). Cobalamin metabolism is closely intertwined with that of folate. For example, cobalamin is required for the activity of MTR, a folate-related enzyme. Decreased cobalamin can
- 5 affect folate metabolism through the folate trap [Rosenblatt, In: *The Metabolic and Molecular Bases of Inherited Disease*, Scriver *et al.* (eds), New York: McGraw-Hill, pp. 3111-3128 (1995); Quadros *et al.*, *Biochem. Biophys. Res. Commun.*, **222**:149-154 (1996)].

TABLE 4

COBALAMIN-RELATED GENES/ENZYMES/TRANSPORTERS*

	Cobalamin-Related Genes/Enzymes/Transporters*	SEQ ID NO:
5	(gastric) intrinsic factor, GIF, MIM-261000 (combined deficiency of GIF & R-binder, MIM 243320	31
	intrinsic factor receptor, IFCR, MIM-261100	
	transcobalamin I, TCI (an R-protein, plasma), MIM 189905	32
10	transcobalamin III, TCIII (an R-protein, plasma), MIM-none	
	other R-proteins (R-binders, cobalophylins, haptocorrins), MIM 193090	
	transcobalamin II, TCII MIM 275350	33
	transcobalamin II receptor, TCII receptor, MIM-none	
15	methylmalonyl Co-A mutase, MCM (MUT locus), MIM 251000	34
	cblF, lysosomal cbl efflux, MIM 277380	
	cblC, cytosolic cbl metabolism, MIM 277400	
	cblD, cytosolic cbl metabolism, MIM 277410	
	cblA, mitochondrial cbl reduction, (AdoCbl synthesis only), MIM 251100	
20	cblB, cob(I)alamin adenosyltransferase, (AdoCbl synthesis only), MIM 251110	
	cblE, methyltransferase-associated cbl utilization, MIM 236270	
	cblG, methyltransferase-associated cbl utilization, MIM 250940	
	*listed with alternate names, abbreviations, and MIM numbers	

TABLE 5

LOCALIZED GENE LOCI RELATED TO COBALAMIN METABOLISM

Gene/enzyme/transport protein	Location	References
MCM (MUT locus)	6p21.2-p21.1	Qureshi <i>et al.</i> (1994) *
5 IF/GIF	11q12-q13	Hewit <i>et al.</i> (1991) *
TCI (an R-protein, plasma)	11q11-q12.3	Johnston <i>et al.</i> , (1992) Sigal <i>et al.</i> , (1987), *
TCII	22q11.2-q13 22q12/13 border	Li <i>et al.</i> , (1995)
10	notes: MCM=methymalonyl Co-A mutase; IF/GIF=(gastric) intrinsic factor; TCI=transcobalmin I; TCII=transcobalamin II. Location information from GDB (*), from MIM (**). Qureshi <i>et al.</i> , <i>Crit. Rev. Oncol. Hematol.</i> 17:133-151 (1994) Hewit <i>et al.</i> , <i>Genomics</i> 10:432-440 (1991) Johnston <i>et al.</i> , <i>Genomics</i> 12:459-464 (1992) Sigal <i>et al.</i> , <i>N. Engl. J. Med.</i> 317:1330-1332 (1987) 15 Li <i>et al.</i> , <i>Biochem. Biophys. Res. Comm.</i> 208:756-764 (1995)	

Genes of Pyridoxine Metabolism: Pyridoxine metabolism is also complex with three dietary forms convertible to pyridoxal phosphate [Whyte *et al.*, *Hypophosphatasia*, In: The Metabolic and Molecular Bases of Inherited Disease, Scriver *et al.* (eds), New York: McGraw-Hill pp. 4095-4111 (1995)] and many pyridoxine-related and

20 pyridoxine-dependent enzymes including decarboxylases and all aminotransferases (Table 6). A number of pyridoxine-related enzymes have been cloned and localized to a chromosomal region (Table 7). Pyridoxine metabolism is related to folate metabolism, especially 1-carbon transfer reactions: both serine

hydroxymethyltransferases and the P-protein (glycine decarboxylase) of the glycine

25 breakdown system are pyridoxine-dependent.

TABLE 6SOME PYRIDOXINE-RELATED GENES/ENZYMES^a

	1.	cystathionine beta-synthase, CBS,	MIM 236200
	2.	gamma-cystathionase,	MIM 219500
5		(L-cystathionine cysteine-lyase, deaminating), LCCL	
	3.	glycine cleavage system (GCS): glycine decarboxylase (P-protein)	
	4.	serine hydroxymethyltransferase 1, SHMT1,	MIM 182144
	5.	serine hydroxymethyltransferase 2, SHMT2,	MIM 138450
10	6.	kynureninase	MIM 278600
	7.	all aminotransferases,	MIM 258870
		(e.g. ornithine-gamma-aminotranferases, OAT,)	
	8.	decarboxylases,	MIM 266100
15		e.g. glutamic acid decarboxylases, GAD1, GAD2,	
	9.	pyridoxamine(pyridoxine)-5'-phosphate oxidase	MIM 603287

^alisted with alternate names, abbreviations, and MIM numbers.

TABLE 7SOME LOCALIZED GENE LOCI RELATED TO PYRIDOXINE METABOLISM

<u>Gene/enzyme</u>	<u>Location</u>	<u>References</u>
1. GAD2	2q31,	Bu et al., 1992)
5 2. GCS P-protein	9p13	Hamosh et al.1995)
3. GAD1	10p11.23	Bu et al.1992)
4. OAT	10q26	**
5. SHMT2	12q12-14	Garrow et al., 1993; Law and Kao, 1979
10 6. LCCL	16pter-qter	*, **
7. SHMT1	17p11.2	Garrow et al.1993 * **
8. CBS	21q22.3	Munke et al.1988
9. PNPO (PPO)		Ngo et al. 1998

*listed with alternate names, abbreviations, and MIM numbers.

15 Location information from GDB (*), from MIM (**).

notes: GAD2=glutamic acid decarboxylase 2, 67 kDa. GCS=glycine cleaving system,
P-protein=glycine decarboxylase subunit. GAD1=glutamic acid decarboxylase 1,
65 kDa. OAT=ornithine-gamma-aminotranferases. SHMT2=serine
hydroxymethyltransferase 2, mitochondrial. LCCL=gamma-cystathionase
20 (L-cystathionine cysteine-lyase (deaminating). SHMT1=serine
hydroxymethyltransferase 1, soluble. CBS=cystathionine beta-synthase. PNPO=
pyridoxamine(pyridoxine)-5'-phosphate oxidase

References:

- Bu et al., *Proc. Nat. Acad. Sci.*, **89**:2115 (1992).
- 25 Hamosh et al., In: "The Metabolic and Molecular Bases of Inherited Disease",
Scriver et al. (eds), New York: McGraw-Hill pp.1337-1348 (1995).
- Garrow et al. *J. Biol. Chem.* **268**:11910-11916 (1993).
- Law and Kao, *Cytogenet Cell Genet*, **24**: 102-114 (1979).
- Munke et al. *Am J. Hum. Gen.* **42**:550-559 (1988).
- 30 Ngo et al. *Biochemistry* **37**:7741-7748 (1998).

Relevance of Folate, Cobalamine, And Pyridoxine to Schizophrenia: There is considerable evidence that schizophrenia results, at least in part, from damage to brain development *in utero* that becomes symptomatic in late adolescence or early adulthood. The etiology of schizophrenia has both genetic and environmental components. Because folate, cobalamin, and pyridoxine are all ingested and metabolized, they could potentially be both environmental and genetic factors for schizophrenia. Folate, cobalamin, and pyridoxine are relevant to schizophrenia in important ways. First, all of them are required for cell division because of their role in nucleic acid synthesis [Rosenblatt, In: *The Metabolic and Molecular Bases of Inherited Disease*, Scriver *et al.* (eds) New York: McGraw-Hill, pp. 3111-3128 (1995); Benton and Rosenberg, In: *The Metabolic and Molecular Bases of Inherited Disease*, Scriver *et al.* (eds), New York: McGraw-Hill, 3129-3149 (1995)]. The developmental brain insult implicated in schizophrenia [Akbarian *et al.*, *Arch. Gen. Psychiatry*, **50**:169-177 (1993); Akbarian *et al.*, *Arch. Gen. Psychiatry*, **50**:178-187 (1993)] is an abnormality of neurogenesis and neuronal migration, which are midtrimester events requiring cell division. Thus folate, cobalamin, and pyridoxine deficiencies could result in the widespread decreased grey matter volume observed in schizophrenia.

Individuals that become schizophrenic later in life are more likely to be born during the winter and early spring [Boyd *et al.*, *Schizophr. Bull.*, **12**:173-186 (1986); Kendell and Adams, *Br. J. Psychiatry*, **158**:758-763 (1991); O'Callaghan *et al.*, *Br. J. Psychiatry*, **158**:764-769 (1991)]; this corresponds to midtrimester in late fall & winter. Many folate- and pyridoxine-containing foods, *e.g.* dark green leafy vegetables, are less readily available in late fall & winter in northern climates. Seasonality was found to be a major determinant of micronutrient status including folate status in a population of pregnant and lactating women in The Gambia where folate deficiency was widespread [Bates *et al.* *Eur. J. Clin. Nutr.* **48**:660-668 (1994)]. Dietary cobalamin comes from animal foods, *e.g.* meat, dairy products, and fish, and prolonged dietary insufficiency is required to produce cobalamin deficiency unless a

person is a strict vegetarian or already has subclinical deficiency [Sanders and Reddy, *Am. J. Clin. Nutr.*, **59**:1176S-1181S (1994)]. In fact, a significant fraction of the population already has subclinical deficiency for folate [Lewis *et al.*, *Ann. NY Acad. Sci.*, **678**:360-362 (1993)] and for [Carmel *et al.*, *Arch. Intern. Med.*, **147**:1995-1996 (1987); Pennypacker *et al.*, *J. Am. Geriatr. Soc.*, **40**:1197-1204 (1992); Naurath *et al.*, *Lancet.*, **346**:85-89 (1995); Allen *et al.*, *Am. J. Clin. Nutr.*, **62**:1013-1019 (1995); Black *et al.*, *J. Nutr.*, **124**:1179-1188 (1994)]. Also, the dietary folate requirement increases during pregnancy [Scholl *et al.*, *Am. J. clin. Nutr.*, **63**:520-525 (1996); McPartlin *et al.*, *Lancet.*, **341**:148-149 (1993)] and most women become folate
10 deficient during late pregnancy [Giles, *J. Clin. Pathol.*, **19**:1-11 (1966)]. Cobalamin deficiency is also common during pregnancy [Gadowsky *et al.*, *J. Adolesc. Health*, **16**:465-474 (1995)] although subnormal levels of vitamin B12 during pregnancy must be interpreted with caution [Metz *et al.*, *Am. J. Hemetol.*, **48**:251-255 (1995)]. An increase in schizophrenia births has also been noticed after winter famine [Susser and
15 Lin, *Arch. Gen. Psychiatry*, **49**:983-988 (1992)]; Susser *et al.*, *Arch. Gen. Psychiatry*, **53**:25-31 (1996)], a time when severe dietary deficiency of both folate and cobalamin is more likely. A temporary increase in the incidence of neural tube defects was reported in Jamaica 11-18 months following Hurricane Gilbert and was found to be associated with decreased dietary folate [Duff and Cooper, *Am J. Pub.Health* **84**:473-
20 476 (1994)].

Schizophrenia is also associated with obstetrical complications, e.g. low birth weight and prematurity [Lewis and Murray, *J. Psychiatr. Res.*, **21**:413-421 (1987)]. Low birthweight and prematurity have also been associated with dietary folate deficiency during pregnancy Scholl *et al.*, *Am. J. clin. Nutr.*, **63**:520-525 (1996).
25 Hyperhomocysteinemia is a risk factor for unexplained recurrent early pregnancy loss [Wouters *et al.*, *Fertil. Steril.*, **60**:820-825 (1993)] and for abruptio placentae [Goddijn-Wesel *et al.*, *Eur. J. Obstet. Gynecol. Reprod. Biol.*, **66**:23-29 (1996)]. Hyperhomocysteinemia may be related to defects in folate-, cobalamin-, or pyridoxine-dependent reactions [Naurath *et al.*, *Lancet.*, **346**:85-89 (1995)].

Interestingly, stillbirths and schizophrenia share a similar seasonality of birth excess [Torrey *et al.*, *Schizophr. Bull.*, 19:557-562 (1993)]. Also N₂O, an anaesthetic gas that inhibits MTR, a cobalamin-requiring enzyme of folate metabolism, is a reproductive toxin for both men and women [Louis-Ferdinand, *Adverse Drug React. Toxicol Rev.*, 13:193-206 (1994)]. Methotrexate, an inhibitor of dihydrofolate reductase (DHFR), induces abortion.

Dietary folate deficiency and low plasma folate are common in inner city urban populations [Scholl *et al.*, *Am. J. clin. Nutr.*, 63:520-525 (1996)]. Likewise, schizophrenia has been reported to be more common in inner city urban populations [Fuller and Bowler, *Schizophr. Bull.*, 16:591-604 (1990)]. Also, both low folate intake [Schorah and Wild, *Lancet.*, 341:1417 (1993)] and schizophrenia [Dohrenwied *et al.*, *Science*, 255:946-952 (1992)] are correlated with lower socioeconomic status.

Immune function is impaired in folate deficiency [LeLeiko and Chao, In: *Rudolph's Pediatrics*, 20th ed., Stamford, CT: Appleton & Lange, pp. 1001-1010 (1996)], in cobalamin deficiency [Hitzig *et al.*, *Ciba. Found. Symp.*, 68:77-91 (1978)] and in pyridoxine deficiency [Trakatellis *et al.* *Postgrad Med. J.* 73:617-622 (1997)] and deficient individuals are more susceptible to infection. Methotrexate, an inhibitor of dihydrofolate reductase, inhibits immune function [Hughes, In: *Rudolph's Pediatrics*, 20th ed., Stamford, CT: Appleton and Lange, pp. 517-519 (1997)]. And, as mentioned, dietary folate and cobalamin requirements increase during pregnancy [Scholl *et al.*, *Am. J. clin. Nutr.*, 63:520-525 (1996); McPartlin *et al.*, *Lancet.*, 341:148-149 (1993)]. This is relevant because the season-of-birth effect just mentioned in connection with dietary folate, or cobalamin deficiency has also been explained by *in utero* infectious illness, the "viral theory" of schizophrenia. Individuals born following winters with severe influenza epidemics are more likely to develop schizophrenia [Adams *et al.*, *Br. J. Psychiatry*, 163:522-534 (1993)] though not all studies find this effect. Although it has not been demonstrated that either the schizophrenia fetus or the pregnant mother actually developed influenza, the

histologic pattern in schizophrenia of a neuronal migration abnormality during brain development has been seen as compatible with a fetal viral infection [Kovelman and Scheibel, *Biol. Psychiatry*, 19:1601-1621 (1984); Bogerts *et al.*, *Arch. Gen. Psychiatry*, 42:784-791 (1985); Akbarian *et al.*, *Arch. Gen. Psychiatry*, 50:169-177 (1993); Akbarian *et al.*, *Arch. Gen. Psychiatry*, 50:178-187 (1993)]. Thus folate or cobalamin, deficiency during pregnancy could result in greater susceptibility to viral infection affecting mother, fetus, or both. The infectious agent could be influenza itself. Alternatively, a severe influenza epidemic could be a "marker" of a severe winter, and infection by another agent could cause the brain damage. In this way, folate or cobalamin deficiency could cause the season-of-birth effect either through the mechanism of dietary deficiency alone, through maternal immune deficiency and infection, or both.

Methotrexate, a DHFR inhibitor, is also an important therapeutic agent for rheumatoid arthritis. Rheumatoid arthritis has repeatedly been found to have a decreased frequency in schizophrenics, a puzzling finding that remains unexplained [Eaton *et al.*, *Schizophr. Res.*, 6:181-192 (1992)].

The developmental model of schizophrenia postulates that brain damage sustained in the second trimester of fetal life results in schizophrenia later in development [Brixey *et al.*, *J. Clin. Psychol.*, 49:447-456 (1993)]. Both folate and cobalamin are already known to contribute to a first trimester fetal nervous system malformation, spina bifida cystica [Kirke *et al.*, *Q. J. Med.*, 86:703-708 (1993); Gordon, *Brain Dev.*, 17:307-311 (1995)], and possibly other birth defects [Shaw *et al.*, *Lancet.*, 346:393-396 (1995); Czeizel, *Lancet.*, 345:932 (1995)]. Some studies [Whitehead *et al.*, *Q. J. Med.*, 88:763-766 (1995); van der Put *et al.*, *Lancet.*, 346:1070-1071 (1995); Ou *et al.*, *Am. J. Med. Genet.*, 63:610-614 (1996); Chatkupt *et al.*, *Am. Acad. Neurol. Works in Progres*, WIP4: (1996)] suggest that a genetic susceptibility factor for spina bifida is a common allele of the folate gene, MTHFR, the nucleotide 677C->T transition converting an alanine residue to valine resulting in a heat-labile enzyme protein.

Homozygotes for this allele, about 10% of the normal population, have lower erythrocyte folate and plasma folate during pregnancy [Molloy *et al.*, *Lancet.*, 349:1591-1593 (1997)]. Homozygotes for this allele also develop moderately elevated blood homocysteine [van der Put *et al.*, *Lancet.*, 346:1070-1071 (1995); Frosst *et al.*, *Nature Genet.*, 10:111-113 (1995)] in the presence of dietary folate deficiency. Moderate hyperhomocysteinemia is toxic to adults [Fermo *et al.*, *Ann. Intern. Med.*, 123:747-753 (1995)], and toxic to the fetus in early gestation [Wouters *et al.*, *Fertil. Steril.*, 60:820-825 (1993)], and possibly teratogenic in the first trimester causing neural tube defects [Whitehead *et al.*, *Q. J. Med.*, 88:763-766 (1995); van der Put *et al.*, *Lancet.*, 346:1070-1071 (1995); Ou *et al.*, *Am. J. Med. Genet.*, 63:610-614 (1996)]. Thus, the MTHFR heat-labile mutation, in the presence of decreased dietary folate in midtrimester, could be teratogenic both through hyperhomocysteinemia and also through folate deficiency causing the developmental brain damage hypothesized in the developmental model of schizophrenia [Brixey *et al.*, *J. Clin. Psychol.*, 49:447-456 (1993)]. A second common polymorphism of MTHFR, the nt1298 A->C mutation could also be a genetic risk factor for spina bifida [van der Put *et al.*, *Lancet.*, 346:1070-1071 (1995)].

Schizophrenia is a common disorder, affecting 1% or more of the population [Karno *et al.*, In: *Comprehensive Textbook of Psychiatry*/VI, 6th ed., Baltimore: Williams & Wilkins, pp. 902-910 (1995)]. Thus, if a significant proportion of schizophrenia shares a common etiology, both the genetic susceptibility factors and the environmental factors must be common in the population. As mentioned earlier, a significant fraction of the population is already sub-clinically deficient for folate and for cobalamin; also, pregnancy may increase this fraction since dietary folate and cobalamin requirements increase during that time. Several functional polymorphic alleles of folate and cobalamin genes are also common in the population including the MTHFR mutations just mentioned and polymorphisms of thymidylate synthase [Horie *et al.*, *Cell Struct. Funct.*, 20:191-197 (1995)], transcobalamin II [Li *et al.*, *Biochim. Biophys. Acta.*, 1219:515-520 (1994)], and folate-binding proteins [Li *et al.*,

- 1994, *supra*; Shen *et al.*, *Biochem.*, 33:1209-1215 (1994)]. Metabolic indicators of folate or cobalamin deficiency, e.g. hyperhomocysteinemia and hypermethylmalonicacidemia, are also common in the population [Naurath *et al.*, *Lancet.*, 346:85-89 (1995)]. Thus there exists a statistical basis for the hypothesis
- 5 that schizophrenia is a birth defect resulting from the action during gestation of genetic risk factors and environmental factors related to folate and/or cobalamin that lead to the generation of risk factors. Such factors are sufficiently common that at least in principle all cases of schizophrenia could result from this mechanism.
- Finally, folate, cobalamin, and pyridoxine are relevant for schizophrenia because of
- 10 findings in patients. Severe genetic deficiency of MTHFR may cause a "schizophrenia" phenotype [Freeman *et al.*, *N. Engl. J. Med.*, 292:491-496 (1975); Regland *et al.*, *J. Neural Transm. Gen. Sect.*, 98:143-152 (1994)]. Genetic deficiency of other folate and cobalamin enzymes has been reported to cause nervous system disease, psychiatric disease, or schizophrenia-like illness [Mudd *et al.*, In: *The*
- 15 *Metabolic and Molecular Bases of Inherited Disease*, Scriver *et al.* (eds), New York: McGraw-Hill pp. 1279-1327 (1995); Hitzig *et al.*, *Ciba. Found. Symp.*, 68:77-91 (1978); Cooper and Rosenblatt, *Annu. Rev. Nutr.*, 7:291-320 (1987); Shevall and Rosenblatt, *Can. J. Neurol. Sci.*, 19:472-486 (1992); Hall, *Br. J. Haematol.*, 80:117-120 (1992)]. Likewise, dietary deficiencies of folate or cobalamin may have similar
- 20 effects [Cooper and Rosenblatt, *Annu. Rev. Nutr.*, 7:291-320 (1987); Shevall and Rosenblatt, *Can. J. Neurol. Sci.*, 19:472-486 (1992)]. Methylfolate therapy reportedly improved the clinical status of schizophrenics with borderline or definite folate deficiency [Godfrey *et al.*, *Lancet.*, 2:392-395 (1990); Procter, *Br. J. Psychiatry*, 159:271-272 (1991)] although the improvement claimed was small and the finding
- 25 controversial. Folate deficiency has been associated with disturbances in mood [Shulman, In: *Folic Acid in Neurology, Psychiatry, and Internal Medicine*, New York: Raven Pr., 463-474 (1979)], and it has been suggested that the most common neuropsychiatric system abnormality in severe folate deficiency is depression [Reynolds *et al.*, *Lancet.*, ii:196-198 (1984)]. Methyltetrahydrofolate reportedly

- improved symptoms of depression in an open trial in elderly depressed patients [Guaraldi *et al. Ann.Clin.Psychiatry* 5:101-105 (1993)]. Schizophrenics are reported to have an 80% excess mortality from cardiovascular disease [Gottesman, *Schizophrenia Genesis*, Schizophrenia Genesis: The Origins of Madness, W.H. Freeman & Co. N.Y.(1991)]; hyperhomocysteinemia, dietary folate deficiency and the MTHFR 677C->T mutation have been implicated in cardiovascular disease in some studies [Morita *et al., Circulation*, 95:2032-2036 (1997)] but not others (Anderson *et al., J. Am. Coll. Cardiol.* 30:1206-1211 (1997)). Also, kynureninase, an important enzyme of tryptophan metabolism, affecting niacin metabolism and serotonin synthesis, is pyridoxine-dependent. Niacin deficiency (pellagra) can cause mental changes including psychosis and hallucinations [Wilson, *Vitamin deficiency and excess*, pp.472-480. In: *Harrison's Principles of Internal Medicine*, (Scriber *et al.* e's.) McGraw-Hill, Inc., N.Y. (1994)]. Also, clozapine, resperidone, and olanzapine are thought to exert their antipsychotic effect in schizophrenia in part through serotonin receptor antagonism.

Gene Localization Studies in Schizophrenia and Folate/Cobalamine/Pyridoxine

- Genes:* If folate, cobalamin, or pyridoxine genes are susceptibility factors for schizophrenia, it is possible that gene localization studies have already identified candidate chromosome regions that contain such a gene (Tables 3, 5, and 7). For three folate or cobalamin genes, DHFR, TCNII and TYMS, there is excellent concordance with schizophrenia gene localization studies.

- On chromosome 5, DHFR has been located at 5q11.2-13.2. A schizophrenia translocation [t(1;5)(1q32.3;5q11.2-13.3)] was reported [McGillivray *et al., Am. J. Med. Genet.*, 35:10-13 (1990); Bassett, *Br. J. Psychiatry*, 161:323-334 (1992)] affecting 5q11.2-5q13.3. A proband and uncle, both with schizophrenia and eye-tracking abnormalities, had partial trisomy for 5q11.2-5q13.3; the third copy was inserted at 1q32.3 giving a derivative chromosome, der(1)inv ins(1;5)(q32.2;q13.3q11.2). The proband's mother had a balanced translocation but

was phenotypically normal without schizophrenia or eye-tracking abnormalities. She had the derivative chromosome 1 with extra material from chromosome 5 inserted but a corresponding deletion in one of her chromosomes 5. She thus had only two copies of 5q11.2-5q13.3. Further studies [Gilliam *et al.*, *Genomics*, 5:940-944 (1989)]
5 showed that the DHFR gene is located within this deleted region, 5q11.2-13.3. Another schizophrenia chromosome abnormality, inv5(p13;q13), has been reported [Bassett, *Br. J. Psychiatry*, 161:323-334 (1992)] affecting 5q13.

On chromosome 5, two-point lod scores of 4.64 and 2.29 were found [Sherrington *et al.*, *Nature*, 336:164-167 (1988)] for the polymorphic markers D5S76 and D5S39
10 respectively in the region of the chromosome abnormality just discussed [McGillivray *et al.*, *Am. J. Med. Genet.*, 35:10-13 (1990); Bassett, *Br. J. Psychiatry*, 161:323-334 (1992)] affecting 5q11.2-13.3. Two other linkage studies found small positive lod scores in this region [Coon *et al.*, *Biol. Psychiatry*, 34:277-289 (1993); Kendler and Diehl, *Schizophr. Bull.*, 19:261-285 (1993)], but numerous other studies excluded this
15 region under the assumptions and models used [Kendler and Diehl, *Schizophr. Bull.*, 19:261-285 (1993)].

On chromosome 18, TYMS has been located at 18p11.32-p11.22. A ring chromosome with deletion of 18pter-p11,18q23-qter [Bassett, *Br. J. Psychiatry*, 161:323-334 (1992)] was reported in a kindred with schizophrenia and bipolar illness
20 [Bassett, *Br. J. Psychiatry*, 161:323-334 (1992)]. Deletion of a segment of 18p was reported in a schizophrenia chromosome [Bassett, *Br. J. Psychiatry*, 161:323-334 (1992)].

On chromosome 22, TCNII has been located at 22q11.2-q13, possibly at the 22q12/13 border. High lod scores have consistently been obtained in the region of TCNII:
25 IL2RB, in 22q12-q13.1 gave a lod score [Pulver *et al.*, *Am. J. Med. Genet.*, 54:3-43 (1994)] of 2.82. Other markers over a broad region of 22q have given suggestive lod scores. D22S278, in 22q12, gave a lod score [Vallada *et al.*, *Am. J. Med. Genet.*,

60:139-146 (1995)] of 1.51. CRYB2, in 22q11.2-q12.1, gave a lod score [Lasseter *et al.*, *Am. J. Med. Genet.*, 60:172-173 (1995)] of 1.71. D22S10, in 22q11.1-q11.2, gave a lod score [Coon *et al.*, *Biol. Psychiatry*, 34:277-289 (1993)] of 0.79. Highly significant p-values for non-parametric analyses have also been obtained: D22S278, in 22q12, for example gave $p=.001$ [Gill *et al.*, *Am. J. Med. Genet.*, 67:40-45 (1996)].

The deletions of velocardiofacial (VCF) syndrome and related disorders (DiGeorge syndrome (DGS) and CATCH22) are located [Lindsay *et al.*, *Genomics*, 32:104-112 (1996)] at 22q11.2. A psychotic disorder develops in about 10% of patients with VCF syndrome [Chow *et al.*, *Am. J. Med. Genet.*, 54:107-112 (1994)]. TCNII is not known to be located at or within these deletions. VCF and related disorders are relatively uncommon compared to schizophrenia; only 2 of 100 randomly selected patients (92 schizophrenics, 5 with schizoaffective disorder, and 3 with schizophreniform disorder) in the Maryland Epidemiological Sample were found [Lindsay *et al.*, *Am. J. Hum. Genet.*, 56:1502-1503 (1995)] to have VCF-related deletions (and later VCF syndrome) on 22q11.2. Consequently, it is not clear whether schizophrenia linkage studies are detecting a haplotype related to a VCS locus or some other locus in this region, such as TCNII.

For some other folate, cobalamin, or pyridoxine relevant genes, physical or genetic studies of schizophrenia have identified chromosomal regions near the gene.

20

DISCUSSION

The folate-cobalamin hypothesis for schizophrenia is attractive because it suggests that a single mechanism of genetic and environmental factors may play a major role in the etiology and pathogenesis of schizophrenia. The combined result of this mechanism is to damage fetal development, especially brain development by inhibiting nucleic acid synthesis, by affecting gene methylations, by increasing susceptibility to infection, and/or by producing teratogens.

This mechanism addresses several puzzling features of schizophrenia such as the season of birth effect, the association with famine and influenza epidemics, the negative association with rheumatoid arthritis, the associations with obstetrical abnormalities, social class, and urban environment. The mechanism also suggests
5 approaches to diagnostic testing, to prevention, and to improved therapy.

It is not excluded that such a mechanism could also apply to a number of common human developmental disorders that have been shown to have a genetic component to their etiology but whose mode of inheritance has been difficult to determine and for which linkage studies have met with unexpected difficulties or have achieved limited
10 success. These developmental disorders include Tourette's syndrome & related disorders (e.g. obsessive-compulsive disorder and chronic multiple tics syndrome) [Pauls, *Adv Neurol*, **58**:151-157 (1992); McMahon *et al.*, *Adv Neurol*, **58**:159-165 (1992); Heutink *et al.*, *Am J Hum Genet*, **57**:465-473 (1995); Grice *et al.*, *Am J Hum Genet*, **59**:644-652 (1996)], learning disorders, including dyslexia [Lewis, *et al.*,
15 *Behav Genet*, **23**:291-297 (1993); Pennington, *J Child Neurol 10 Suppl*, **1**:S69-S77 (1995)], conduct disorder [Lombroso *et al.*, *J. Am. Acad. Child Adolesc. Psychiatry*, **33**:921-938 (1994)], attention-deficit hyperactivity disorder [Lombroso *et al.*, 1994, *J. Am. Acad. Child Adolesc. Psychiatry*, **33**:921-938 (1994)], bipolar illness [Baron, *Acta. Psychiatr. Scand.*, **92**:81-86 (1995); Benjamin and Gershon, *Biol. Psychiatry*,
20 **40**:313-316 (1996); Risch and Botstein, *Nature Genet.*, **12**:351-353 (1996); Jamison and McInnis, *Nature Med.*, **2**:521-522 (1996); Morell, *Science*, **272**:31-32 (1996)], autism [Lombroso *et al.*, 1994, *J. Am. Acad. Child Adolesc. Psychiatry*, **33**:921-938 (1994)], and obsessive-compulsive disorder in adults [Lombroso *et al.*, 1994, *J. Am. Acad. Child Adolesc. Psychiatry*, **33**:921-938 (1994)]. Some of these disorders have
25 been shown to be associated with schizophrenia.

The present invention may be better understood by reference to the following non-limiting Examples, which are provided as exemplary of the invention. The following Examples are presented in order to more fully illustrate one embodiment of the

invention. They should in no way be construed, however, as limiting the broad scope of the invention.

EXAMPLE 1

DIAGNOSING SCHIZOPHRENIA

5 Structure of Datafiles

Data are arranged in a file suitable for input into a binary logistic regression program (Table 8). A model is created consisting of those explanatory variables actually available from the specific patient-to-be-diagnosed and family members participating in the testing. This new combined data set (reference data set + data from patient-to-be-diagnosed with participating family members) is analyzed by binary logistic regression for the model chosen giving the predicted probability that a proband is affected with schizophrenia for all of the probands including the patient-to-be-diagnosed.

The model can be modified if required. The goodness of fit for the patient-to-be-diagnosed is checked. The predicted probability that the patient-to-be-diagnosed has schizophrenia is compared with a classification table generated from the model used to determine likelihood of false positives and false negatives. The predicted probability that the patient-to-be-diagnosed is affected with schizophrenia, with likelihood of false positive or false negative result, is returned to the clinician.

TABLE 8
A HYPOTHETICAL PARTIAL REFERENCE DATA SET OF GENETIC
EXPLANATORY VARIABLES TO ILLUSTRATE DATA STRUCTURE

ID	resp	P111	P112	P211	P212	M111	M112	M311	F511	S2-411	CA1-111
1	1	1	0	1	1	1	1	0	0	1	1
2	1	1	0	0	0	0	0	0	1	0	0
3	1	1	1	1	0	1	0	0	1	1	1
4	1	0	0	0	0	0	0	1	0	0	0
5	1	0	0	1	1	1	1	0	0	0	1
6	0	1	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	1	0	0	0
8	0	0	0	1	0	0	0	0	1	1	0
9	0	1	0	0	0	1	0	0	0	1	1
10	0	0	0	0	0	1	0	0	0	1	0
11	...										
n											

For each proband (Table 8), the record contains several variables:

identification number (ID) of the proband.

a binary response variable (resp) for affection status of the proband: response=1, if the proband is affected with schizophrenia; response=0 if proband is unaffected (*i.e.* a control individual). The proband is not necessarily one of the individuals for whom genotype data (explanatory variables) are available. The patient-to-be-diagnosed is assigned response=0 when added to the reference data set.

a set of explanatory variables: *i.e.* sets of genotypes of mutations found in the schizophrenia patients and family members and controls and family members. The schizophrenia patients and the control individuals are probands (P) as is the patient-to-be-diagnosed. Unaffected family members are the proband's mother (M), father (F), sib(s) (S1, S2, etc.), child(ren) (C1, C2, etc.) or other relatives. Data for affected family members, *e.g.* the proband's mother (MA), father (FA), sibs (SA1, SA2, etc.), children (CA1, CA2, etc.), or other relatives, are entered as separate explanatory variables.

Genetic explanatory variables: Each individual has 0, 1, or 2 copies of any given mutation allele at a given locus. Thus a genotype at each locus contributes two independent explanatory variables. Most of the affected family members will be relatives of schizophrenia probands, but occasionally a relative of an unaffected proband will turn out to be affected with schizophrenia.

Mutations are tabulated as explanatory variables: (see Table 8):

- (i) by the proband or relative in whom they occur, (e.g. P, M, F, S2, C1, MA, FA, SA1, CA1, other);
- (ii) by the specific folate, cobalamin, or pyridoxine gene locus in which they occur (e.g. 1=DHFR locus, 2=MTHFR locus, 3=TCN2 locus, 4=MTR locus, 5=CBS locus, etc.);
- (iii) by the specific mutation within a locus (e.g., 1=the first-designated mutation within a locus, 2=the second-designated mutation within a locus, etc.); and
- (iv) by whether the individual has a single or double dose of the mutation. Thus an explanatory variable P321 records whether the proband has a single dose of the second-designated mutation of the third-designated locus, *i.e.* TCN2. A variable M312 records whether the proband's mother has a double dose of the first-designated TCN2 mutation studied.

In the present hypothetical reference dataset illustrated of genetic explanatory variables (Table 8), partial genotype data for probands, mothers, fathers, sibs and children are given for five gene loci. Not all of the possible explanatory variables are shown. Probands 1-5 are unrelated individuals with the definite clinical diagnosis of schizophrenia; probands 6-10 are unrelated unaffected (control) individuals. Probands 1, 2, 3, 6 and 9 all have a single copy of the first-designated DHFR mutation; proband 3 also has a second copy of that mutation. Probands 1, 3, 5 and 8 all have a single copy of the first-designated mutation at the MTHFR locus; probands 1 and 5 also have a second copy of that mutation. Mothers of probands 1, 3, 5, 9 and 10 all have a single copy of the first-designated DHFR mutation; mothers of probands

- 1 and 5 also have a second copy of this mutation. Mothers of probands 4 and 7 each have a single copy of the first-designated mutation of TCN2; data for a double dose are not shown. The fathers of probands 2, 3, and 8 each have a single copy of the first designated mutation of CBS; data for a double dose are not shown. The second
- 5 (unaffected) sibs of probands 1, 3, 8, 9, and 10 each have a single copy of the first-designated mutation of MTR; data for a double dose are not shown. The first affected children of probands 1, 3, 5, and 9 each have a single copy of the first-designated mutation of DHFR. Other susceptibility loci and mutations can be incorporated in Table 8 in the same fashion *e.g.*, cytokine gene mutations or
- 10 polymorphisms, or major histocompatibility complex (MHC) mutations or polymorphisms.

Environmental explanatory variables: If only genetic explanatory variables (genotype data) are used, the maximum predicted probability that the proband is affected with schizophrenia is expected to be approximately about 0.5 in most

15 populations. When environmental risk factors are included as explanatory variables, the maximum predicted probability that the proband is affected with schizophrenia may approach 1.0. Examples of environmental risk factors for a schizophrenia patient include:

- (1) the proband's dietary folate/cobalamin/pyridoxine intake.
- 20 (2) the proband's circulating levels of folate/cobalamin/pyridoxine.
- (3) the proband's circulating levels of homocysteine, methylmalonic acid, or cystathionine. Elevated levels are indicators of subtle folate/cobalamin deficiency.
- (4) the proband's mother's dietary folate/cobalamin/pyridoxine intake at the time of patient diagnosis, during a pregnancy, or during the pregnancy that produced the
- 25 proband.
- (5) the proband's mother's circulating levels of homocysteine, methylmalonic acid, or cystathionine at the time of patient diagnosis, during a pregnancy, or during the pregnancy that produced the proband.

- (6) dietary or circulating folate/cobalamin/pyridoxine or circulating levels of homocysteine, methylmalonic acid, or cystathionine for other family members.
- (7) epidemiological factors related to the proband's gestation and birth, *e.g.* low birth weight or preterm birth, maternal infection, maternal smoking (associated with
- 5 low plasma folate), season of birth (late winter or spring births are more common in schizophrenia), etc.

Method of Data Analysis

The method exemplified herein is based upon the published guide for the SAS system, but other software can be used. The dataset is analyzed using binary logistic

10 regression to model the response probability, p_i , that the i th proband's affection status is 1, *i.e.* the probability that the i th proband has schizophrenia, given the vector of explanatory variables, x_i . That is:

$$p_i = \text{Prob}(y_i=1|x_i).$$

To do this the logit transformation of p_i is modeled as a linear function of the

15 explanatory variables in the vector, x_i :

$$\text{logit}(p_i) = \log(p_i/[1-p_i]) = \alpha + \beta'x_i$$

where: α is the intercept parameter and

β is the vector of slope parameters.

In SAS, the "descending" option is used to model the probability that the response=1,

20 as in the present analysis, rather than response=0.

Outputs of binary logistic regression analysis

After analysis of a dataset, the outputs obtained from SAS include:

- (a) Estimates and standard errors of the parameters (α and β).

Using estimates of the intercept parameter (α) and the slope parameter (β) for

25 each environmental or genetic risk factor, the logistic regression equation for the dataset can be written.

(b) Significance tests of the parameters (*e.g.* Wald chi-square). From the corresponding p -values, the level of significance of each of the environmental or

genetic risk factors is determined. A global significance test of the data with corresponding p-value is also determined.

(c) Odds ratios are given for the slope parameters of each environmental or genetic risk factor. Thus the amount contributed by each environmental or genetic risk factor to the risk of schizophrenia is determined.

(d) The confidence limits for regression parameters and odds ratios are determined.

(e) The predicted probabilities of the observations can be computed, *i.e.* the probability that each individual in the dataset has schizophrenia:

10 $\alpha\sim$ = estimate of the intercept parameter;

$\beta\sim$ = vector of the estimates of the slope parameters;

x = vector of the explanatory variables;

$p\sim$ = predicted probabilities

15
$$p\sim = \frac{1}{1 + \exp(\alpha\sim - \beta\sim x)}$$

(f) The model is modified by adding or removing variables until a model is found that best fits the data;

(g) The model is tested for goodness-of-fit. Also, the degree of influence of each specific observation is tested to detect extreme or ill-fitting observations. These may be examples of data entry errors or alternatively, observations that do not fit the present model for schizophrenia.

(h) The probability that a new individual (the patient-to-be-diagnosed) is schizophrenic is then calculated from the final, modified, best fitting regression equation based upon parameters derived from a corrected/modified data set. A simple method of doing this is to add the data for the patient-to-be-diagnosed to the reference data set, a large group of well-studied schizophrenia probands, schizophrenia family members, control probands and control family members for whom data are available for many explanatory variables. A model is created consisting of those informative explanatory variables actually available from the specific patient-to-be-diagnosed and family members participating in the testing. This new combined data set (reference

data set + data from patient-to-be-diagnosed with participating family members) is analyzed by binary logistic regression for the model chosen giving the predicted probability that a proband is affected with schizophrenia for all of the probands including the patient-to-be-diagnosed.

- 5 (i) A classification table is produced from the data set by the "jack knifing" procedure or an approximation to it. This procedure classifies each observation as an event or nonevent based on the model that omits the observation being classified. A classification table sorts observations into percent correct, percent false positives, and percent false negatives at various probability levels and computes
- 10 sensitivity and specificity.

- (j) The data set used for diagnostic testing is constantly being updated and the regression equation corrected. For example, stratification by geographic residence or geographic origin of ancestors must be considered for some environmental or genetic risk factor.
- 15 For example, in Table 9, entries 34-43 are shown for the data file containing genotypes of 38 schizophrenic probands plus 211 control probands; the first 38 are the affected probands. For individual 302088, the proband is affected ("1"); there is a single dose ("1") of the DHFR mutation but not a double dose ("0") and a single dose ("1") of the MTHFR mutation but not a double dose ("0"). The number 302088
- 20 identifies the individual whose genotypes are listed; the proband, in this case, is the same individual.

TABLE 9SAS DATAFILE FOR SCHIZOPHRENIA PATIENTS AND CONTROLS

	...						
	...						
5	34	302086	1	1	0	1	1
	35	302088	1	1	0	1	0
	36	302110	1	1	0	1	0
	37	302111	1	1	0	0	0
	38	302136	1	1	1	1	0
10	39	100001	0	1	0	0	0
	40	100061	0	0	0	0	0
	41	100064	0	1	0	1	0
	42	100067	0	0	0	1	0
	43	100073	0	1	0	0	0
15	...						
	...						
	...						
	...						

In Table 10, entries 31-40 are shown for the data file containing genotypes of 35
 20 mothers of schizophrenic probands plus (the same) 211 control probands. For
 individual 302083, the proband is affected ("1"); there is a single dose of the DHFR
 mutation ("1") but not a double dose ("0"); there is neither a single ("0") nor a double
 ("0") dose of the MTHFR mutation. The number 302083 identifies the individual
 whose genotypes are listed, a mother; the proband, in this case, is a different
 25 individual, her affected child.

TABLE 10SAS DATAFILE FOR SCHIZOPHRENIA MOTHERS AND CONTROLS

	...						
	...						
5	31	302083	1	1	0	0	0
	32	302103	1	0	0	1	0
	33	302104	1	0	0	1	0
	34	302105	1	1	0	1	0
	35	302120	1	0	0	0	0
10	36	100001	0	1	0	0	0
	37	100061	0	0	0	0	0
	38	100064	0	1	0	1	0
	39	100067	0	0	0	1	0
	40	100073	0	1	0	0	0
15	...						
	...						

In Table 11, entries 11-20 are shown for the data file containing genotypes of 15 fathers of schizophrenic probands plus (the same) 211 control probands. For individual 302084, the proband is affected ("1"); there is a single dose ("1") but not a double dose ("0") of the DHFR mutation; there is both a single ("1") and a double dose ("1") of the MTHFR mutation. The number 302084 identifies the individual whose genotypes are listed, a father; the proband, in this case, is a different individual, his affected child.

TABLE 11SAS DATAFILE FOR SCHIZOPHRENIA FATHERS AND CONTROLS

	...						
	...						
5	11	302102	1	0	0	0	0
	12	302106	1	1	0	0	0
	13	302115	1	1	0	0	0
	14	302117	1	1	0	0	0
	15	302084	1	1	0	1	1
10	16	100001	0	1	0	0	0
	17	100061	0	0	0	0	0
	18	100064	0	1	0	1	0
	19	100067	0	0	0	1	0
	20	100073	0	1	0	0	0
15	...						
	...						

In Table 12, entries 9-18 are shown for the data file containing genotypes of 13 unaffected sibs of schizophrenic probands plus (the same) 211 control probands. For individual 302089, the proband is affected ("1"); there is a single dose ("1") but not a double dose ("0") of the DHFR mutation; there is both a single ("1") and a double dose ("1") of the MTHFR mutation. The number 302089 identifies the individual whose genotypes are listed, an unaffected sib; the proband, in this case, is a different individual, the affected sib of individual 302089.

TABLE 12SAS DATAFILE FOR SCHIZOPHRENIA SIBS AND CONTROLS

...							
5	...						
	09	302071		1	1	0	0 0
	10	302073	1	0	0	1	0
	11	302089	1	1	0	1	1
	12	302118	1	1	0	0	0
10	13	302126	1	1	0	0	0
	14	100001	0	1	0	0	0
	15	100061	0	0	0	0	0
	16	100064	0	1	0	1	0
	17	100067	0	0	0	1	0
15	18	100073	0	1	0	0	0
...							
...							

In Tables 9-12 for individual 100061, the proband is unaffected ("0"); there is neither a single dose ("0") nor a double dose ("0") of the DHFR mutation; there is neither a

20 single dose ("0") nor a double dose ("0") of the MTHFR mutation. Since the proband is unaffected, this is a control individual. The number 100061 identifies the individual whose genotypes are listed, as a control individual; the proband, in this case, is the same individual. The identical group of control individuals is used for all four comparisons.

EXAMPLE 2Distribution of Folate Gene Polymorphism Genotypes Among Schizophrenics,
Schizophrenia Parents, Schizophrenia Sibs, and ControlsSummary

- 5 The DNA polymorphism-Diet-Cofactor-Development hypothesis (DDCD hypothesis, described above) postulates that schizophrenia results in part from developmental brain damage sustained *in utero* from the aggregate effect of maternal defects of genes related to important cofactors, *e.g.* folate, cobalamin, pyridoxine, potentiated by a maternal dietary deficiency of these cofactors. The maternal damage to the fetus
- 10 results in part from insufficiency of these cofactors themselves and in part from resulting effects such as immune deficiency and maternal teratogens, *e.g.* hyperhomocysteinemia. Genes from either parent acting in the fetus may modify these damaging effects as outlined in the gene-teratogen model (described above).

The hypothesis addresses all of the unusual biological and epidemiological features of

15 schizophrenia: *e.g.* the decreased amount of grey matter in brain areas, the unusual birth-month effect, the geographical differences in incidence, the socioeconomic predilection, the association with obstetrical abnormalities (low birth weight and prematurity), the decreased incidence of rheumatoid arthritis, and the association with viral epidemics (described above).

- 20 The hypothesis can be supported by finding significant association of sequence variants of folate, cobalamin, or pyridoxine genes with schizophrenia. Folate, cobalamin, and pyridoxine absorption, transport, and metabolism are complex [Rosenblatt, In: *The Metabolic and Molecular Bases of Inherited Disease*, Scriver *et al.* (eds), New York: McGraw-Hill, pp. 3111-3128 (1995); Benton and Rosenberg, In:
- 25 *The Metabolic and Molecular Bases of Inherited Disease*, Scriver *et al.* (eds), New York: McGraw-Hill, pp. 3129-3149 (1995); Whyte *et al.*, *Hypophosphatasia*, In: *The*

Metabolic and Molecular Bases of Inherited Disease, Scriver *et al.* (eds), New York: McGraw-Hill pp. 4095-4111] with multiple transport proteins, enzymes, and regulatory components. A strong candidate for harboring a mutation predisposing to schizophrenia is the DHFR gene coding for the folate enzyme dihydrofolate

- 5 reductase. DHFR chemically reduces dietary folate converting it into a form that can enter cellular metabolism. DHFR is also important for DNA synthesis and is known to play a major role in development *in utero*. A novel polymorphic 19 basepair deletion of the DHFR gene has been isolated which could be of functional significance because it affects potential transcription factor binding sites.
- 10 A second candidate is the MTHFR gene, coding for methylenetetrahydrofolate reductase, MTHFR, an important enzyme of folate metabolism. MTHFR was of particular interest because severe deficiency of enzyme activity has been associated with the "schizophrenia" phenotype [Freeman *et al.*, *N. Engl. J. Med.*, 292:491-496 (1975); Regland *et al.*, *J. Neural Transm. Gen. Sect.*, 98:143-152 (1994)] and because
- 15 a common mutation, the nt677 C->T transition results in a mutated gene that encodes a heat-labile MTHFR, having decreased enzymatic activity, which in the presence of dietary folate deficiency, causes the plasma homocysteine of homozygotes to become elevated [van der Put *et al.*, *Lancet.*, 346:1070-1071 (1995); Frosst *et al.*, *Nature Genet.*, 10:111-113 (1995)]. In adults, hyperhomocysteinemia is known to cause
- 20 vascular disease and to be toxic [Frosst *et al.*, *Nature Genet.*, 10:111-113 (1995)]. Therefore, homocysteine that crosses the placenta could act as a fetal teratogen during pregnancy. Maternal folate deficiency could also have a more direct teratogenic effect through fetal folate deprivation. These effects could be potentiated by abnormalities of other folate, cobalamin, or pyridoxine genes, even if these
- 25 abnormalities were only minor.

Materials & Methods:

1. *Subjects and Sample Collection:* Patients with schizophrenia and unaffected family members of schizophrenics, were ascertained from patient facilities, patient support

groups, and family support group organizations. Nearly all schizophrenia families had only a single case of schizophrenia. The patients came from different schizophrenia families than the parents and sibs. The controls were unaffected and unrelated individuals not known to be schizophrenic or related to patients with
5 schizophrenia or spina bifida. All subjects were of Caucasian background except two of the schizophrenia patients who were of African American background.

After informed consent was obtained, 20-40 ml of blood was collected into EDTA (purple-top) vacutainers, placed on ice immediately, and transported to the laboratory where plasma, packed red cells, and buffy coat were separated by centrifugation and
10 frozen at -80°C.

2. *Detection of Alleles:* DNA was isolated using the QIAmp column DNA extraction procedure or the QIAGEN Genomic-tip method (QIAGEN, Chatsworth, CA). Alleles for a newly detected polymorphic 19 bp deletion in the dihydrofolate reductase (DHFR) gene were determined by polymerase chain reaction (PCR) amplification of
15 the region surrounding the deletion using specific primers (Fig 1) and direct detection of the PCR products after separation of products on a non-denaturing polyacrylamide gel. A Cetus - Perkin-Elmer 9600 thermocycler was used. Briefly, the PCR reaction contained 200 uM dNTPs, 1.5 mM MgCl₂, 10 pmols of each primer, in 10 ul reaction volume. The PCR conditions used were denaturation at 94°C for 6 min. initially,
20 followed by 35 cycles of 94°C for 55 sec., 60°C for 55 sec., and 72°C for 55 sec. and a final extension at 72°C for 12 min.

Alleles for the 677C->T transition of the methylenetetrahydrofolate reductase (MTHFR) gene were determined by cleavage with the restriction endonuclease, HinfI, of PCR-amplified genomic DNA from blood and separation of the products by
25 non-denaturing polyacrylamide gel electrophoresis [Frosst *et al.*, *Nature Genet.*, 10:111-113 (1995)].

3. *Sequencing the Region Around the DHFR Deletion:* Using the same primers (Figure 1), genomic DNA from individuals with 1,1 and 2,2 genotypes was amplified by PCR and the products sequenced using an ABI PRISM 377 automated sequencer. Restriction sites were identified using the MAP Program in the GCG Package.

- 5 Potential transcription factor binding sites were detected with the TESS program (transcription element search software, URL:<http://agave.humgen.upenn.edu/tess/index.html>).

4. *Data Analysis:* Since the mode of inheritance of schizophrenia is unknown, binary logistic regression was used to test the DHFR deletion allele and the MTHFR

- 10 heat-labile allele as genetic risk factors for schizophrenia. Either the DHFR deletion polymorphism or the MTHFR heat-labile allele could itself be a genetic risk factor for schizophrenia. The genotypes of the two folate gene polymorphisms were used as explanatory variables. Genotypes of schizophrenia patients, parents, or sibs were compared with those of controls.

- 15 Four files were constructed consisting of schizophrenia patients+controls, mothers of schizophrenia patients+controls, fathers of schizophrenia patients+controls, and sibs of schizophrenia patients+controls for input into the SAS System. Each dataset contained 6 variables. In order, these were:

1. six digit identification (ID) number;
- 20 2. response variable, *i.e.* affection status of the proband
(0=unaffected, *i.e.* control individual; 1=affected, *i.e.* schizophrenia patient);
3. DHFR mutation-single dose (Ds);
4. DHFR mutation-double dose (Dd);
5. MTHFR mutation-single dose (Ms); and
- 25 6. MTHFR mutation-double dose (Md).

For mutation data, 0=mutation absent, 1=mutation present.

Results

Alleles of the DHFR 19 bp Deletion Polymorphism: Amplification of the region of intron 1 of DHFR defined by the primers in Figure 1 gave two polymorphic bands of 232 and 213 bp after separation on a non-denaturing polyacrylamide gel (Figure 2).

- 5 Sequencing the PCR products from the two homozygotes showed that they differed by 19 bp (Figure 3). The upper and lower bands (Figure 2), non-deletion allele and deletion allele respectively, were designated alleles 1 and 2 respectively. Comparison with two published sequences showed that allele 1 was identical with one of them [Yang *et al. J. Mol. Biol.* 176:169-187 (1984)] indicating that allele 2 resulted from a
10 19 bp deletion. The other published sequence [Chen *et al. J. Biol. Chem.* 259:3933-3943 (1984)] was lacking one base pair of allele 1, an A indicated by "*" in Fig 3. It is possible that this shorter reference sequence [Chen *et al. J. Biol. Chem.* 259:3933-3943 (1984)] resulted from a sequencing artifact.

- Sequences in the 19 bp Deleted Region of DHFR Intron 1:* The 19bp sequence in the
15 deleted region (Fig 3) of DHFR intron 1 contained sites for several restriction enzymes including RsaI and ScrFI, and potential binding sites for transcription factors including Sp1, NF-kappaB, CP1 (NF-Y), E2F, ETF and GCF in the 19 base-pair region.

- Binary Logistic Regression Analysis:* The number of individuals with each genotype
20 of the two polymorphisms among 38 unrelated schizophrenia probands, 35 unrelated mothers of schizophrenia probands, 15 unrelated fathers of schizophrenia probands, 13 unrelated unaffected sibs of schizophrenia probands, and 211 unrelated unaffected control probands is shown in Table 13.

TABLE 13
DISTRIBUTION OF DHFR AND MTHFR MUTATION GENOTYPES
AND ALLELES AMONG CONTROLS, SCHIZOPHRENICS,
AND SCHIZOPHRENIA FAMILY MEMBERS

5	<u>DHFR 19 bp deletion polymorphism:</u>					
	--GenTyp--	-----Schizophrenia-----				---Ctrl---
		P	M	F	S	
	1/1	6 (.16)	10 (.29)	4 (.27)	4 (.31)	56 (.26)
	1/2	22 (.58)	13 (.37)	11 (.73)	8 (.61)	115 (.54)
10	2/2	10 (.26)	12 (.34)	0 (0.0)	1 (.08)	40 (.19)
	total	38 (1.00)	35 (1.00)	15 (1.00)	13 (1.00)	211 (.99)

<u>MTHFR 677C->T transition polymorphism:</u>						
--GenTyp--		-----Schizophrenia-----				---Ctrl---
		P	M	F	S	
15	1/1	14 (.37)	16 (.46)	11 (.73)	4 (.31)	103 (.49)
	1/2	18 (.47)	18 (.51)	3 (.20)	8 (.61)	78 (.37)
	2/2	6 (.16)	1 (.03)	1 (.07)	1 (.08)	30 (.14)
	total	38 (1.00)	35 (1.00)	15 (1.00)	13 (1.00)	211 (1.00)

P=schizophrenia patients; M=mothers of schizophrenia patients; F=fathers of
 20 schizophrenia patients; S=unaffected sibs of schizophrenia patients; Ctrl=control
 individuals.

The four data files were analyzed using the logistic procedure of SAS (SAS Institute Inc., 1995) and the "descending" option, which modeled the probability that RESPONSE=1, that is, the probability that the proband was affected with schizophrenia. Note that the proband was not always the individual whose genotype data were used. For example, genotype data for mothers of schizophrenic probands were used to determine the probability that their children, the probands, were affected. Use of the "best" model selection options for logistic analysis in SAS gave the best models for two and three explanatory variables, (Table 14).

Table 14BINARY LOGISTIC REGRESSION RESULTSGENETIC RISK FACTORMODEL: Ds Dd Ms Md

Odds Ratio (p value)

Schizophrenia Patients

Ds OR(p)	1.937 (.18)
Dd OR(p)	1.263 (.59)
Ms OR(p)	1.775 (.14)
Md OR(p)	0.914 (.86)

Mothers of Schizophrenia Patients

Ds OR(p)	0.630 (.31)
Dd OR(p)	2.653 (.028)*
Ms OR(p)	1.439 (.34)
Md OR(p)	0.143 (.065)

Fathers of Schizophrenia Patients

Ds OR(p)	1.178 (.79)
Dd OR(p)	0.000 (.96)
Ms OR(p)	0.366 (.14)
Md OR(p)	0.841 (.88)

Unaffected Sibs of Schizophrenia Patients

Ds OR(p)	1.104 (.88)
Dd OR(p)	0.337 (.31)
Ms OR(p)	2.688 (.12)
Md OR(p)	0.317 (.29)

Notes For Table 14DHFR 19 bp deletion:

Ds=single dose;

Dd=double dose

MTHFR 677C->T mutation: Ms=single dose;

Md=double dose

Logistic regression model:

Model with four explanatory variables (Ms, Md, Ds and Dd).

OR(p)=odds ratio and the corresponding p-value for that odds ratio
determination *=significant at the $p \leq .05$ level.

0.000 odds ratios occurred since none of the fathers of schizophrenia patients had genotype Dd; there was a possibly quasi- complete separation in the sample points; the maximum likelihood estimate may not exist; and therefore validity of the model fit for these odds ratios was questionable.

The comparison of mothers of schizophrenia probands with control probands was statistically significant. Ds was not a significant genetic risk factor. Neither Ms nor Md in mothers was a significant genetic risk factor. However, the p-value for Md decreased and approached significance ($p=.065$) at the $p<.05$ level.

- 5 *Predicted Probabilities of the Various Genotypes:* The "probs predicted" modality of SAS, gave the predicted probability that the proband was affected with schizophrenia (response=1) given genotype data for control probands and schizophrenia patients (probands), mothers of schizophrenia probands, fathers of schizophrenia probands, or sibs of schizophrenia probands. The maximum probabilities obtained are shown in
- 10 Table 15. The highest maximum predicted probability that the proband was affected was obtained for genotype data from mothers of schizophrenia probands, next for schizophrenia probands, next for fathers of schizophrenia probands, and lowest for sibs of schizophrenia probands.

TABLE 15
MAXIMUM PREDICTED PROBABILITY

<u>Model</u>	<u>P</u>	<u>M</u>	<u>F</u>	<u>S</u>
Ds Dd Ms Md 0.24	0.29	0.12	0.11	

Model and explanatory variables are the same as in Table 14.

Determination of Genotypes Conferring the Highest Risk: The predicted probabilities that the proband was affected with schizophrenia given specific genotypes of control probands and schizophrenia probands, mothers of schizophrenia probands, fathers of schizophrenia probands, or sibs of schizophrenia probands were determined using the

5 model containing all four explanatory variables (Table 16). The predicted probabilities that the proband was affected with schizophrenia were highest for maternal genotypes (Table 15). The maternal genotype with the highest risk was Dd Ms, conferring a probability of 0.29 of schizophrenia in the proband (Table 16). The Dd Ms genotype also gave the highest predicted probability, 0.24, for schizophrenia

10 patients.

TABLE 16
PREDICTED PROBABILITIES FOR SPECIFIC GENOTYPES

<u>Model: Ds Dd Ms Md</u>			
<u>Genotype</u>	<u>Predicted</u>	<u>Genotype</u>	<u>Predicted</u>
	<u>Probability</u>		<u>Probability</u>
<u>Schizophrenia Patients:</u>			
Dnull + Mnull	0.07	Ds + Ms	0.20
Dnull + Ms	0.12	Ds + Md	0.19
Dnull + Md	0.11	Dd + Ms	0.24
Ds + Mnull	0.12	Dd + Md	0.23
Dd + Mnull	0.15		
<u>Mothers of Schizophrenia Patients:</u>			
Dnull + Mnull	0.16	Ds + Ms	0.13
Dnull + Ms	0.20	Ds + Md	0.02
Dnull + Md	0.03	Dd + Ms	0.29
Dd + Mnull	0.22	Dd + Md	0.06
Ds + Mnull	0.10		
<u>Fathers of Schizophrenia Patients:</u>			
Dnull + Mnull	0.10	Ds + Ms	0.05
Dnull + Ms	0.04	Ds + Md	0.04
Dnull + Md	0.03	Dd + Ms	0.0
Ds + Mnull	0.12	Dd + Md	0.0
Dd + Mnull	0.0		
<u>Unaffected Sibs of Schizophrenia Patients:</u>			
Dnull + Mnull	0.04	Ds + Ms	0.11
Dnull + Ms	0.10	Ds + Md	0.04
Dnull + Md	0.03	Dd + Ms	0.04
Ds + Mnull	0.04	Dd + Md	0.01
Dd + Mnull	0.02		

Genotypes consist of the same explanatory variables described in Table 14 except that Dnull has no copy of the DHFR deletion and Mnull has no copy of the MTHFR 677C->T variant. Odds ratios of 0.0 were unsatisfactory as described in Table 14.

Discussion

Structure and Function of the DHFR 19 bp Deletion Polymorphism: DHFR polymorphisms have been reported previously [Feder *et al.*, *Nucl. Acids Res.* **15**:5906 (1987); Detera-Wadleigh *et al.*, *Nucl. Acids Res.* **17**:6432 (1989)]. It is known that
5 introns are important for message regulation *e.g.*, splicing, or as sites for binding transcription factors. Since the first intron is a relatively common location for regulatory elements, it is possible that the deleted region of DHFR intron 1 could play a role in regulation of DHFR or that the deletion could be a genetic risk factor for schizophrenia because it removes potential transcription factor binding sites.
10 Abnormalities of transcription factors and their binding sites may play a role in disease. For example, a polymorphic Sp1 binding site in the collagen type I alpha 1 gene has been associated with reduced bone density and osteoporosis [Grant *et al.*, *Nature Genet.* **14**:203-205 (1996)].

The Nature of the Putative Folate Genetic Risk Factors for Schizophrenia: Dd in the
15 mother of a schizophrenia proband conferred significantly increased risk of schizophrenia in her child (Table 14). The findings that Dd was a genetic risk factor in mothers but not fathers of schizophrenia probands (Table 15) and that Dd in mothers gave a higher predicted probability than in schizophrenia patients, fathers or sibs (Tables 15 and 16) was consistent with the role of DHFR as a teratogenic locus
20 according to the gene-teratogen model (described above). The finding that a double dose but not a single dose of the DHFR deletion in mothers was a genetic risk factor (Table 16) supported a recessive mode of action in the mother. A teratogenic locus acting in the mother can also act as a modifying or specificity locus in the fetus.

Neither Ms nor Md in mothers of schizophrenia probands showed statistical
25 significance as genetic risk factors for schizophrenia in probands (Table 14). However Md in mothers approached statistical significance ($p=.065$) and appeared to be protective (odds ratio 0.14), while Ms in mothers appeared to increase risk modestly (odds ratio 1.44, $p=.34$).

Role of Genetic and Environmental Factors in Schizophrenia: Since the probability that a schizophrenia co-twin is also affected is reported [Gottesman, *Schizophrenia Genesis*, Schizophrenia Genesis- The Origins of Madness, W.H. Freeman & Co. N.Y.(1991)] to be only 48%, a large part of the risk for schizophrenia would be anticipated to come from environmental factors. Therefore, some controls should have the genetic risk factors for schizophrenia but not be affected with schizophrenia. In the present data set, 6 of 35 schizophrenia mothers and 7 of 38 schizophrenia patients had Dd Ms, the genotype conferring the highest risk, compared with 15 of 211 controls. Since this genotype gave predicted probabilities of schizophrenia in probands of 0.29 and 0.24 respectively, polymorphisms of DHFR and MTHFR could account for a considerable portion of the genetic component of the risk of schizophrenia.

Relation of DHFR to Cytogenetic and Linkage Data for Schizophrenia: As discussed above, the DHFR gene has been located on chromosome 5 at 5q11.2-13.2. A schizophrenia translocation was reported (McGillivray et al.1990; Bassett, 1992) affecting 5q11.2-5q13.3. Also two-point lod scores of 4.64 and 2.29 were found [Sherrington *et al.*, *Nature*, **336**:164-167 (1988)] for the polymorphic markers D5S76 and D5S39 respectively on chromosome 5, in this region [McGillivray *et al.*, *Am. J. Med. Genet.*, **35**:10-13 (1990); Bassett, *Br. J. Psychiatry*, **161**:323-334 (1992)]. Two other linkage studies found small positive lod scores in this region [Coon *et al.*, *Biol. Psychiatry*, **34**:277-289 (1993); Kendler and Diehl, *Schizophr. Bull.*, **19**:261-285 (1993)], but numerous other studies excluded this region under the assumptions and models used [Kendler and Diehl, *Schizophr. Bull.*, **19**:261-285 (1993)]. Recently, new studies have found suggestive evidence for a potential susceptibility locus at a different region of 5q, 5q31 [Schwab *et al.*, *Nat. Genet.* **11**:325-327 (1997)] and 5q22-31 [Straub *et al.*, *Molec Psychiatr.* **2**:148-155 (1997)].

The case-control study presented herein illustrates the usefulness of the DNA polymorphism-Diet-Cofactor-Development and the gene-teratogen models described

above. More importantly, the results presented herein, clearly fail to reject the specific models, *i.e.*, that folate gene polymorphisms can play a role in the etiology of schizophrenia.

The present invention is not to be limited in scope by specific embodiments described
5 herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and the accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

Various publications in addition to the immediately foregoing are cited herein, the
10 disclosures of which are incorporated by reference in their entireties.

We Claim:

1. A method of generating a genetic reference dataset for use in the determination of the predicted probability for an individual of having a susceptibility for a developmental disorder due to genetic factors or for developing a developmental disorder due to genetic factors or for having offspring that develop a developmental disorder due to genetic factors comprising:
 - (a) collecting a biological sample from a human subject; wherein the human subject is selected from the group consisting of a diagnostic proband, a blood relative of the diagnostic proband, an affected proband, a blood relative of the affected proband, a control proband, and a blood relative of the control proband; wherein the biological sample contains nucleic acids and/or proteins from the human subject;
 - (b) analyzing the nucleic acids and/or proteins from the biological sample; wherein said analyzing results in a partial or full genotype for the alleles of the genes involved in folate, pyridoxine, and/or cobalamin metabolism; wherein said partial or full genotype forms a dataset of genetic explanatory variables for the human subject; and
 - (c) compiling the dataset of genetic explanatory variables from multiple human subjects into a genetic reference dataset.
2. A method of generating a genetic and environmental reference dataset for use in the determination of the predicted probability for an individual of having a susceptibility for a developmental disorder due to genetic factors and environmental factors or for developing a developmental disorder due to genetic factors and environmental factors or for having offspring that develop a developmental disorder due to genetic factors and environmental factors comprising:
 - (a) obtaining dietary and epidemiological information for environmental explanatory variables for the human subjects of Claim 1; and

(b) combining said environmental explanatory variables with a genetic reference dataset for the human subjects.

3. The method of Claim 2 wherein the developmental disorder is selected from the group consisting of schizophrenia, spina bifida cystica, Tourette's syndrome,
5 dyslexia, conduct disorder, attention-deficit hyperactivity disorder, bipolar illness, autism, chronic multiple tic syndrome and obsessive-compulsive disorder.

4. A method of generating an environmental reference dataset for use in the determination of the predicted probability for an individual of having a susceptibility for a developmental disorder due to environmental factors or for developing a
10 developmental disorder due to environmental factors or for having offspring that develop a developmental disorder due to environmental factors comprising:

(a) obtaining dietary and epidemiological information for environmental explanatory variables for a human subject; wherein the human subject is selected from the group consisting of a diagnostic proband, a blood relative of the diagnostic
15 proband, an affected proband, a blood relative of the affected proband, a control proband, and a blood relative of the control proband; and

(b) compiling a dataset of environmental explanatory variables from multiple human subjects into an environmental reference dataset for the human subjects.

20 5. A method of estimating the genetic susceptibility of an individual to have or to develop a developmental disorder comprising:

(a) collecting a biological sample from one or more participants; wherein a participant is either the individual or a blood relative of the individual; and wherein the biological sample contains nucleic acids and/or proteins of the participant;

25 (b) analyzing the nucleic acids and/or proteins from the biological sample; wherein said analyzing results in a partial or full genotype for the alleles of the genes

involved in folate, pyridoxine, and/or cobalamin metabolism; and wherein said partial or full genotype forms a dataset of genetic explanatory variables for the participants;

(c) adding the datasets of genetic explanatory variables obtained from steps (a) and (b) to a genetic reference dataset therein forming a combined genetic
5 dataset;

(d) formulating a model comprising the genetic explanatory variables obtained from the participants; and

(e) analyzing the combined genetic dataset; wherein a predicted probability for the individual of having or developing a developmental disorder is
10 determined; and wherein the genetic susceptibility of an individual to have or to develop a developmental disorder is estimated.

6. The method of Claim 5 wherein said analyzing the combined genetic dataset is performed by binary linear regression.

7. The method of Claim 6 further comprising the step of :

15 (f) modifying the model by adding or subtracting a genetic explanatory variable; and re-analyzing the combined genetic dataset by binary logistic regression; wherein a model is chosen that best fits the data.

8. The method of Claim 7 further comprising the step of :

(g) testing the model for goodness of fit.

20 9. The method of Claim 8 wherein the binary linear regression is performed with the SAS system.

10. The method of Claim 5 wherein the developmental disorder is selected from the group consisting of schizophrenia, spina bifida cystica, Tourette's syndrome, dyslexia, conduct disorder, attention-deficit hyperactivity disorder, bipolar illness,
25 autism, chronic multiple tic syndrome and obsessive-compulsive disorder.

11. The method of Claim 10 wherein the developmental disorder is schizophrenia and the individual is suspected of being genetically susceptible of having or for developing schizophrenia.
12. The method of Claim 11 wherein the individual is suspected of being
5 genetically susceptible for having or for developing schizophrenia because a blood relative has schizophrenia.
13. The method of Claim 12 wherein the blood relative is a parent, a sibling, or a grandparent.
14. The method of Claim 13 wherein the blood relative is a parent and wherein the
10 parent is the mother of the individual.
15. A method of estimating the genetic and environmental susceptibility of an individual to have or to develop a developmental disorder comprising:
- (a) collecting a biological sample from one or more participants; wherein a participant is either the individual or a blood relative of the individual; and wherein
15 the biological sample contains nucleic acids and/or proteins of the participant;
 - (b) analyzing the nucleic acids and/or proteins from the biological sample; wherein said analyzing results in a partial or full genotype for the alleles of the genes involved in folate, pyridoxine, and/or cobalamin metabolism; and wherein said partial or full genotype forms a dataset of genetic explanatory variables for the participants;
 - 20 (c) obtaining dietary and epidemiological information for environmental explanatory variables for the participants; wherein said information forms a dataset of environmental explanatory variables for the participants;
 - (d) adding the datasets of genetic explanatory variables obtained from steps (a) and (b) and the dataset of environmental explanatory variables of step (c) to
25 a genetic and environmental reference dataset therein forming a combined genetic and environmental dataset;
 - (e) formulating a model comprising the genetic and environmental explanatory variables obtained from the participants; and

(f) analyzing the combined genetic and environmental dataset by binary logistic regression;

wherein a predicted probability for the individual of having or developing a developmental disorder is determined; and wherein the genetic and environmental susceptibility of an individual to have or to develop a developmental disorder is estimated.

16. The method of Claim 15 further comprising the step of :
(g) modifying the model by adding or subtracting a genetic or environmental explanatory variable; and re-analyzing the combined genetic and environmental dataset by binary logistic regression; wherein a model is chosen that best fits the data.

17. The method of Claim 16 further comprising the step of :
(h) testing the model for goodness of fit.

18. The method of Claim 17 wherein the binary linear regression is performed with the SAS system.

19. A method of estimating the susceptibility of an individual to have offspring that develop a developmental disorder comprising:

(a) collecting a biological sample from one or more participants; wherein a participant is either the individual or a blood relative of the individual; and wherein the biological sample contains nucleic acids and/or proteins of the participant;

(b) analyzing the nucleic acids and/or proteins from the biological sample; wherein said analyzing results in a partial or full genotype for the alleles of the genes involved in folate, pyridoxine, and/or cobalamin metabolism; and wherein said partial or full genotype forms a dataset of genetic explanatory variables for the participants;

(c) adding the datasets of genetic explanatory variables obtained from steps (a) and (b) to a genetic reference dataset therein forming a combined genetic dataset;

(d) formulating a model comprising the genetic explanatory variables obtained from the participants; and

(e) analyzing the combined genetic dataset by binary logistic regression; wherein a predicted probability for the individual to have offspring that
5 develop a developmental disorder is determined; and wherein the genetic and environmental susceptibility of an individual to have offspring that develop a developmental disorder is estimated.

20. The method of Claim 19 further comprising the step of :

(f) modifying the model by adding or subtracting a genetic explanatory
10 variable; and re-analyzing the combined genetic dataset by binary logistic regression; wherein a model is chosen that best fits the data.

21. The method of Claim 20 further comprising the step of :

(g) testing the model for goodness of fit.

22. The method of Claim 21 wherein the binary linear regression is performed
15 with the SAS system.

23. The method of Claim 22 wherein the individual is a pregnant woman.

24. A method of lowering the risk of a pregnant woman who has been determined by the method of Claim 23 to be susceptible to have offspring that develop a developmental disorder comprising administering methylfolate, cobalamin or
20 pyridoxine to the pregnant woman, wherein said administering lowers the risk of the pregnant woman of giving birth to offspring with a developmental disorder.

25. A method of determining if any treatment is advisable for a pregnant woman who has been determined by the method of Claim 23 to be susceptible to having offspring that develop a developmental disorder comprising determining the

concentration of a risk factor from a tissue sample or body fluid from the pregnant woman; wherein when the concentration of the risk factor is statistically above or below an accepted normal range, treatment is advisable.

26. A method of monitoring the effect of the administration of methylfolate, cobalamin or pyridoxine to the pregnant woman of Claim 25, comprising determining the concentration of a risk factor from a tissue sample or body fluid from the pregnant woman; and wherein when the concentration of the risk factor is statistically within an accepted normal range, the treatment is effective.

27. The method of Claim 26 wherein the risk factor is selected from the group consisting of homocysteine, folate, and cobalamin.

28. The method of Claim 22 wherein the individual is the mate of a pregnant woman.

29. A method of treating an asymptomatic individual determined by the method of Claim 23 to be susceptible for developing a developmental disorder comprising administering methylfolate, cobalamin or pyridoxine.

30. An isolated nucleic acid encoding a genetic variant of human dihydrofolate reductase comprising a nucleotide sequence having a 19 base-pair deletion spanning nucleotides 540 to 558 of the nucleotide sequence of SEQ ID NO:41.

31. The isolated nucleic acid of Claim 30 that has the nucleotide sequence of SEQ ID NO:42.

32. An expression vector comprising the nucleic acid of Claim 30 operably associated with an expression control sequence, wherein the nucleic acid is selected from the group consisting of cDNA or genomic DNA.

33. A PCR primer that can be used to distinguish SEQ ID NO:42 from the nucleotide sequence selected from the group consisting of SEQ ID NO:41 and SEQ ID NO:45.
34. The PCR primer of Claim 33 that comprises 10 to 50 consecutive nucleotides
5 from the nucleotide sequence selected from the group of SEQ ID NO: 41, the complementary strand of SEQ ID NO: 41, SEQ ID NO:42, the complementary strand of SEQ ID NO: 42, SEQ ID NO:45, and the complementary strand of SEQ ID NO: 45.
35. The PCR primer of Claim 34 wherein the 10 to 50 consecutive nucleotides are
10 from nucleotides 350 to 530 of SEQ ID NO:41.
36. The PCR primer of Claim 35 having the nucleotide sequence of 5'-CTA AAC TGC ATC GTC GCT GTG-3' (SEQ ID NO:38).
37. The PCR primer of Claim 36 wherein the 10 to 50 consecutive nucleotides are from the complementary strand of nucleotides 550 to 850 of SEQ ID NO:41.
- 15 38. The PCR primer of Claim 37 having the nucleotide sequence of 5'-AAA AGG GGA ATC CAG TCG G-3' (SEQ ID NO:39).
39. An isolated nucleic acid that hybridizes under standard hybridization conditions to a nucleic acid having the nucleotide sequence ACCTGGGCGGGACGCGCCA (SEQ ID NO:40) or a sequence complementary to
20 SEQ ID NO:40; wherein said isolated nucleic acid consists of 12 to 48 nucleotides.
40. An isolated nucleic acid that hybridizes to the nucleotide sequence of SEQ ID NO:42, but not to the nucleotide sequence of SEQ ID NO:41; when said hybridizing is performed under identical conditions.

41. An isolated nucleic acid that hybridizes to the complementary strand of the nucleotide sequence of SEQ ID NO:42, but not to the complementary strand of the nucleotide sequence of SEQ ID NO:41; when said hybridizing is performed under identical conditions.
- 5 42. An isolated nucleic acid that hybridizes to the nucleotide sequence of SEQ ID NO:41, but not to the nucleotide sequence of SEQ ID NO:42; when said hybridizing is performed under identical conditions.
43. An isolated nucleic acid that hybridizes to the complementary strand of the nucleotide sequence of SEQ ID NO:41, but not to the complementary strand of the
10 nucleotide sequence of SEQ ID NO:42; when said hybridizing is performed under identical conditions.
44. The method of Claim 5 wherein said analyzing the nucleic acids and/or proteins from the biological sample comprises determining if the biological sample contains a genetic variant of human dihydrofolate reductase having a nucleotide
15 sequence with a 19 base-pair deletion spanning nucleotides 540 to 558 of the nucleotide sequence of SEQ ID NO:41; and wherein the genetic variant of human dihydrofolate reductase is an explanatory variable.
45. The method of Claim 44 wherein said determining is performed by a method selected from the group consisting of PCR, special PCR, RT PCR, RFLP analysis,
20 SSCP, and FISH.
46. The method of Claim 1 wherein said analyzing the nucleic acids and/or proteins from the biological sample comprises determining if the biological sample contains the genetic variant of human dihydrofolate reductase having a nucleotide sequence with a 19 base-pair deletion spanning nucleotides 540 to 558 of the
25 nucleotide sequence of SEQ ID NO:41; and wherein the genetic variant of human dihydrofolate reductase is an explanatory variable.

47. The method of Claim 46 wherein said determining is performed by a method selected from the group consisting of PCR, special PCR, RT PCR, RFLP analysis, SSCP, and FISH.

Primers for PCR Amplification the DHFR Deletion Polymorphism Region

Forward primer: 5'-CTA AAC TGC ATC GTC GCT GTG-3'

Reverse primer: 5'-AAA AGG GGA ATC CAG TCG G-3'

Figure 1

Genotypes of the DHFR 19 bp Deletion
by Non-denaturing Polyacrylamide Gel Electrophoresis

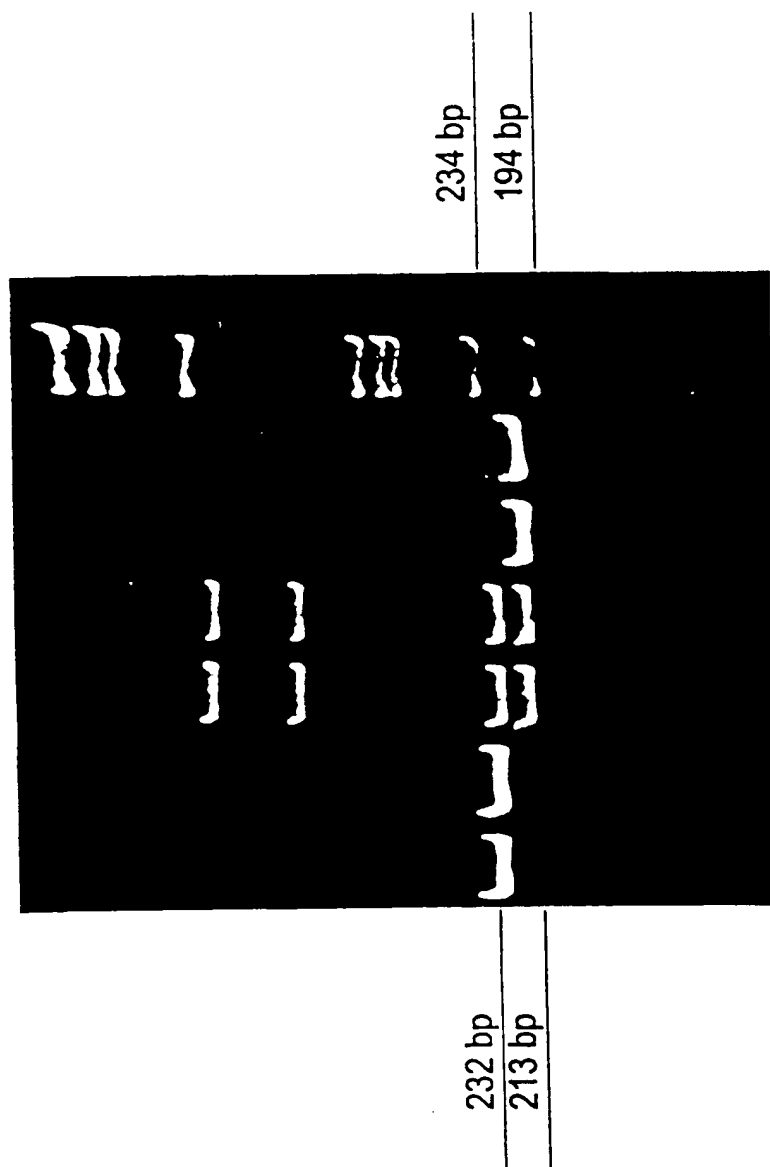


Figure 2

Sequences of PCR Amplification Products
in the Region of the DHFR Deletion Polymorphism Region

Allele 1 GCTGCCCAACGGTCGGGGTACCCTGGGCGGGAACGCGCCAGGCGGACTCCCGGCGAGA
 ||||| |||||
Allele 2 GCTGCCCAACGGTCGGGGT.....GGCGGACTCCCGGCGAGA

Figure 3

```
1 CTGCAGCGCC AGGGTCCACC TGGTCGGCTG CACCTGTGGA GGAGGAGGTG
51 GATTTTCAGGC TTCCCGTAGA CTGGAAGAAT CGGCTCAAAA CCGCTTGCCT
101 CGCAGGGGCT GAGCTGGAGG CAGCGAGGCC GCCCGACGCA GGCTTCCGGC
151 GAGACATGGC AGGGCAAGGA TGGCAGCCCCG GCGGCAGGGC CCGGCGAGGA
201 GCGCGAACCC GCGGCCGAG TTCCCAGGCG TCTGCGGGCG CGAGCACGCC
251 GCGACCCTGC GTGCGCCGGG GCGGGGGGGC GGGGCCTCGC CTGCACAAAT
301 AGGGACGAGG GGGCGGGGCG GCCACAATT CGCGCCAAAC TTGACCGCGC
351 GTTCTGCTGT AACGAGCGGG CTCGGAGGTC CTCCCGCTGC TGTCATGGTT
401 GGTTGCTAA ACTGCATCGT CGCTGTGTCC CAGAACATGG GCATCGGCAA
451 GAACGGGGAC CTGCCCTGGC CACCGCTCAG GTATCTGCCG GGCCGGGGCG
501 ATGGGACCCA AACGGGCGCA GGCTGCCAC GGTGCGGGTA CCTGGGCGGG
551 ACGCGCCAGG CCGACTCCCG GCGAGAGGAT GGGGCCAGAC TTGCGGTCTG
601 CGCTGGCAGG AAGGGTGGGC CCGACTGGAT TCCCTTTTC TGCTGCGCGG
651 GAGGCCCAGT TGCTGATTTT TGCCCGGATT CTGCTGCCCC GTGAGGTCTT
701 TGCCCTGCGG CGCCCTCGCC CAGGGCAAAG TCCCAGCCCT GGAGAAAACA
751 CCTCACCCCT ACCCACAGCG CTCCGTTTGT CAGGTGCCTT AGAGCTCGAG
801 CCCAAGGGAT AATGTTTCGA GTAACGCTGT TTCTCTAACT TGTAGGAATG
851 AATTGAGATA TTTCCAGAGA ATGACCACAA CCTCTTCAGT AGAAGGTAAT
901 GTGGGATTAA GTAGGGTCTT GCTTGATGAA GTTTACCAGT GCAAATGTTA
951 GTTAAATGGA AAGTTTCCG TGTTAATCTG GGACCTTTTC TCTTATTATG
1001 GATCTGTATG ATCTGTATGC AGTTCCCAAG GTTCATTTAC CATTATTAAA
1051 AAATTTTTGT CTTAGAAATT TTATGTATGT CAACGCACGA GCAAATTATC
1101 AGGCATGGGG CAGAATTGGC AACTGGGTGG AGGCTTCGGT GGAGGTTAGC
1151 ACTCCGAAAG GAAAACAGAG TAGGCCTTTG GAACAGCTGC TGAAGAGAT
1201 AAGGCCTGAA CAAGGGCAGT GGAGAAGAGA GGGTAAAAAT TTTTAAAGGT
1251 TACATGACCC TGGATTTTGG AGATC
```

Figure 4A

```

1 CTGCAGCGCC AGGGTCCACC TGGTCGGCTG CACCTGTGGA GGAGGAGGTG
51 GATTTTCAGGC TTCCCGTAGA CTGGAAGAAT CGGCTCAAAA CCGCTTGCCT
101 CGCAGGGGCT GAAGCTGGAGG CAGCGAGGCC GCCCGACGCA GGCTTCCGGC
151 GAGACATGGC AGGGCAAGGA TGGCAGCCCG GCGGCAGGGC CCGGCAGAGGA
201 GCGCGAACCC GCGGCCGCAG TTCCAGGGCG TCTGCGGGCG CGAGCAGCC
251 GCGACCTGCG GTGCGCCGGG GCGGGGGGGC GGGGCCTCGC CTGCACAAAT
301 AGGGACGAGG GGGCGGGGCG GCCACAATTT CGCGCCAAAC TTGACCGCGC
351 GTTCTGCTGT AACGAGCGGG CTCGGAGGTC CTCCCGCTGC TGTCATGGTT
401 GGTTTCGTAA ACTGCATCGT CGCTGTGTCC CAGAACATGG GCATCGGCAA
451 GAACGGGGAC CTGCCCTGGC CACCGCTCAG GTATCTGCCG GGCCGGGGCG
501 ATGGGACCCA AACGGGCGCA GGCTGCCCCAC GGTCGGGGT
551 GG CCGACTCCCG GCGAGAGGAT GGGGCCAGAC TTGCGGTCTG
601 CGCTGGCAGG AAGGGTGGGC CCGACTGGAT TCCCCTTTTC TGCTGCGCGG
651 GAGGCCAGT TGCTGATTTT TGCCCGGATT CTGCTGCCCG GTGAGGTCTT
701 TGCCCTGCGG CGCCCTCGCC CAGGGCAAAG TCCCAGCCCT GGAGAAAACA
751 CCTCACCCCT ACCCACAGCG CTCCGTTTGT CAGGTGCCTT AGAGCTCGAG
801 CCCAAGGGAT AATGTTTCGA GTAACGCTGT TTCTCTAACT TGTAGGAATG
851 AATTCAGATA TTTCCAGAGA ATGACCACAA CCTCTTCAGT AGAAGGTAAT
901 GTGGGATTAA GTAGGGTCTT GCTTGATGAA GTTTACCAGT GCAAATGTTA
951 GTTAAATGGA AAGTTTCCG TGTTAATCTG GGACCTTTTC TCTTATTATG
1001 GATCTGTATG ATCTGTATGC AGTTCCCAAG GTTCATTTAC CATTATTAAA
1051 AAATTTTGT CTTAGAAATT TTATGTATGT CAACGCACGA GCAAATTATC
1101 AGGCATGGGG CAGAATTGGC AACTGGGTGG AGGCTTCGGT GGAGGTTAGC
1151 ACTCCGAAAG GAAAACAGAG TAGGCCTTTG GAACAGCTGC TGGAAGAGAT
1201 AAGGCCTGAA CAAGGGCAGT GGAGAAGAGA GGTAAAAAT TTTTAAAGGT
1251 TACATGACCC TGGATTTTGG AGATC

```

Figure 4B

SEQUENCE LISTING

<110> Johnson, William G.
Stenroos, Edward S.

<120> METHODS FOR DIAGNOSING, PREVENTING, AND TREATING
DEVELOPMENTAL DISORDERS

<130> 601-1-057PCT

<140> UNASSIGNED

<141> 2000-05-24

<150> UNASSIGNED

<151> 2000-05-23

<150> 60/136,198

<151> 1999-05-25

<160> 46

<170> PatentIn Ver. 2.0

<210> 1

<211> 2187

<212> DNA

<213> Homo sapiens

<400> 1

```

gccatggtga acgaagccag aggaaacagc agcctcaacc cctgcttggg gggcagtgcc 60
agcagtggca gtgagagctc caaagatagt tcgagatggt ccaccccggg cctggaccct 120
gagcggcatg agagactccg ggagaagatg aggcggcgat tggaatctgg tgacaagtgg 180
ttctccctgg aattcttccc tcctcgaact gctgagggag ctgtcaatct catctcaagg 240
tttgaccgga tggcagcagg tggccccctc tacatagacg tgacctggca cccagcaggt 300
gaccctggct cagacaagga gacctctccc atgatgatcg ccagcaccgc cgtgaactac 360
tgtggcctgg agaccatcct gcacatgacc tgctgccgtc agcgccctga ggagatcacg 420
ggccatctgc acaaagctaa gcagctgggc ctgaagaaca tcatggcgct gcggggagac 480
ccaataggtg accagtggga agaggaggag ggaggcttca actacgcagt ggacctgggtg 540
aagcacatcc gaagtgagtt tggtgactac tttgacatct gtgtggcagg ttaccccaaa 600
ggccaccccc aagcagggag ctttgaggct gacctgaagc acttgaagga gaaggtgtct 660
gcgggagccg atttcatcat cacgcagctt ttctttgagg ctgacacatt cttccgcttt 720
gtgaaggcat gcaccgacat gggcatcact tgccccatcg tccccgggat ctttcccatc 780
cagggctacc actcccttcg gcagcttgtg aagctgtcca agctggaggt gccacaggag 840
atcaaggacg tgattgagcc aatcaaagac aacgatgctg ccatccgcaa ctatggcatc 900
gagctggccg tgagcctgtg ccaggagctt ctggccagtg gcttggtgcc aggcctccac 960
ttctacaccc tcaaccgcga gatggctacc acagaggtgc tgaagcgccg ggggatgtgg 1020
actgaggacc ccaggcgctc cctaccctgg gctctcagtg cccaccccaa gcgccgagag 1080
gaagatgtac gtcccatctt ctgggcctcc agaccaaaga gttacatcta ccgtacccag 1140
gagtgggacg agttccctaa cgcccgctgg ggcaattcct cttcccttgc ctttggggag 1200
ctgaaggact actacctctt ctacctgaag agcaagtccc ccaaggagga gctgctgaag 1260
atgtgggggg aggagctgac cagtgaagca agtgtctttg aagtctttgt tctttacctc 1320

```

```

tcgggagaaac caaacccggaa tggtcacaaa gtgacttgcc tgccttgga c gatgagccc 1380
ctggcggtctg agaccagcct gctgaaggag gagctgctgc ggggtgaaccg ccagggcatc 1440
ctcaccatca actcacagcc caacatcaac ggggaagccgt cctccgaccc catcgtgggc 1500
tgggggcccca gcgggggcta tgtcttccag aaggcctact tagagttttt cacttcccgc 1560
gagacagcgg aagcatttct gcaagtgtctg aagaagtacg agctccgggt taattaccac 1620
cttgtcaatg tgaagggtga aaacatcacc aatgcccctg aactgcagcc gaatgtgtgc 1680
acttggggca tcttccctgg gcgagagatc atccagccca ccgtagtga tcccgtcagc 1740
ttcatgttct ggaaggacga ggcctttgcc ctgtggattg agcgggtggg aaagctgtat 1800
gaggaggagt ccccgctccg caccatcatc cagtacatcc acgacaacta ctctctggtc 1860
aacctggtgg acaatgaact cccactggac aactgcctct ggcaggtggg ggaagacaca 1920
ttggagcttc tcaacaggcc caccagaat gcgagagaaa cggaggctcc atgaccctgc 1980
gtcctgacgc cctgcgttgg agccactcct gtcccgctt cctcctccac agtgcgtgct 2040
ctcttgggaa ctccactctc ctctgtgtct cccccaccc ggctccact cccccacctg 2100
acaatggcag ctgactgga gtgaggcttc caggctcttc ctggacctga gtcggcccca 2160
catgggaacc tagtactctc tgctcta 2187

```

<210> 2

<211> 7122

<212> DNA

<213> Homo sapiens

<400> 2

```

gcgcgtgtct ggctgctagg ccgacaccaa ggactggccg ggtacccggg aagaaagcac 60
gtgtctccagc agttgccgcg cccagccccg agagaggccc tagggcgctg cgggctttcg 120
gggtccgcag tccccccgcg acgcgagcca acgggaggcg tcaaaagacc cgggccttgc 180
gtggcaggct cgcctggcgc tggctggcgt ggcccttggc cgtcgtcacc tgtggagagc 240
acgtcttctc tgccgcgcc tctgcgcaag gaggagaetc gacaacatgt cccccgcgt 300
ccaagacctg tcgcaacccg aaggtctgaa gaaaacctg cgggatgaga tcaatgccat 360
tctgcagaag aggtattatg tgctggatgg agggatgggg accatgatcc agcgggagaa 420
gctaaacgaa gaacacttcc gaggtcagga atttaaagat catgccaggc cgctgaaagg 480
caacaatgac attttaagta taactcagcc tgatgtcatt taccaaatcc ataaggaata 540
cttgctggct ggggcagata tcattgaaac aaatactttt agcagcacta gtattgcccc 600
agctgactat ggccttgaac acttggccta ccggatgaac atgtgctctg caggagtggc 660
cagaaaagct gccgaggagg taactctcca gacaggaatt aagaggtttg tggcaggggc 720
tctgggtccg actaataaga cactctctgt gtcccatct gtggaaaggc cggattatag 780
gaacatcaca tttgatgagc ttgttgaagc ataccaagag caggccaaag gacttctgga 840
tgggcggggt gatattctac tcattgaaac tatttttgat actgccaatg ccaaggcagc 900
cttgtttgca ctccaaaatc tttttgagga gaaatatgct ccccgcccta tctttatttc 960
agggacgac gttgataaaa gtgggcggac tctttccgga cagacaggag agggatttgt 1020
catcagcgtg tctcatggag aaccactcta cattggatta aattgtgctt tgggtgcagc 1080
tgaaatgaga ccttttattg aaataattgg aaaaatgtaca acagcctatg tcctctgtta 1140
tcccaatgca ggtcttccca acacctttgg tgactatgat gaaacgcctt ctatgatggc 1200
caagcaccta aaggattttg ctatggatgg cttggtcaat atagttggag gatgctgtgg 1260
gtcaaacacca gatcatatca gggaaattgc tgaagctgtg aaaaattgta agcctagagt 1320
tccacctgcc actgcttttg aaggacatat gttactgtct ggtctagagc ccttcaggat 1380
tggaaccgtac accaactttg ttaacattgg agagcgctgt aatgttgag gatcaaggaa 1440
gtttgtctaaa ctcatcatgg caggaaacta tgaagaagcc ttgtgtgttg ccaaagtgc 1500
gggtggaaatg ggagccaggt gtgtggatgt caacatggat gatggcatgc tagatggtcc 1560
aagtgcattg accagatttt gcaacttaat tgcttccgag ccagacatcg caaaggtacc 1620
tttgtgcatt gactcctcca attttgctgt gattgaagct ggggttaaagt gctgccaaag 1680
gaagtgcatt gtcaatagca ttagtctgaa ggaaggagag gacgacttct tggagaaggc 1740

```

caggaagatt	aaaaagtatg	gagctgctat	ggtggtcatg	gcttttgatg	aagaaggaca	1800
ggcaacagaa	acagacacaa	aatcagagt	gtgcaccgg	gcctaccatc	tgcttgtgaa	1860
aaaactgggc	tttaatccaa	atgacattat	ttttgaccct	aatatccctaa	ccattgggac	1920
tggaatggag	gaacacaact	tgtatgccat	taattttatc	catgcaacaa	aagtcattaa	1980
agaaacatta	cctggagcca	gaataagtgg	aggtctttcc	aacttgctct	tctccttccg	2040
aggaatggaa	gccattcgag	aagcaatgca	tggggttttc	ctttaccatg	caatcaagtc	2100
tggcatggac	atggggatag	tgaatgctgg	aaacctccct	gtgtatgatg	atatccataa	2160
ggaacttctg	cagctctgtg	aagatctcat	ctggaataaa	gaccctgagg	ccactgagaa	2220
gctcttacgt	tatgcccgag	ctcaaggcac	aggagggaag	aaagtcattc	agactgatga	2280
gtggagaaat	ggccctgtcg	aagaacgcct	tgagtatgcc	cttgatgaag	gcattgaaaa	2340
acataattatt	gaggatactg	aggaagccag	gttaaaccac	aaaaaatatc	cccgacctct	2400
caatataaatt	gaaggacccc	tgatgaatgg	aatgaaaatt	gttggtgatc	tttttggagc	2460
tggaataatg	tttctacctc	aggttataaa	gtcagccggg	gttatgaaga	aggctgttgg	2520
ccaccttatc	cctttcatgg	aaaaagaaaag	agaagaaacc	agagtgcctta	acggcacagt	2580
agaagaagag	gacctttacc	agggcacccat	cgtgctggcc	actgttaaag	gcgacgtgca	2640
cgacataggc	aagaacatag	ttggagtagt	ccttggctgc	aataatttcc	gagttattga	2700
tttaggagtc	atgactccat	gtgataagat	actgaaagct	gctcttgacc	acaaagcaga	2760
tataattggc	ctgtcaggac	tcatcactcc	ttccctggat	gaaatgattt	ttgttgccaa	2820
ggaaatggag	agattagcta	taaggattcc	attgttgatt	ggaggagcaa	ccacttcaaa	2880
aaccacacaca	gcagttaaaa	tagctccgag	atacagtgca	cctgtaatcc	atgtcctgga	2940
cgctccaag	agtgtggtgg	tgtgttccca	gctgttagat	gaaaatctaa	aggatgaata	3000
ctttgaggaa	atcatggaag	aatatgaaga	tattagacag	gaccattatg	agtctctcaa	3060
ggagaggaga	tacttaccct	taagtcaagc	cagaaaaagt	ggtttccaaa	tggattggct	3120
gtctgaacct	caccacgtga	agcccacgtt	tattgggacc	caggtctttg	aagactatga	3180
cctgcagaag	ctggtggact	acattgactg	gaagcctttc	tttgatgtct	ggcagctccg	3240
gggcaagtac	ccgaatcgag	gctttcccaa	gatattttaac	gacaaaacag	taggtggaga	3300
ggccagggaag	gtctacgatg	atgccacaaa	tatgctgaac	acactgatta	gtcaaaagaa	3360
actccggggc	cgggggtgtg	ttgggttctg	gccagcacag	agtatccaag	acgacattca	3420
cctgtacgca	gaggctgctg	tgccccaggc	tgcagagccc	atagccacct	tctatggggt	3480
aaggcaacag	gctgagaagg	actctgccag	cacggagcca	tactactgcc	tctcagactt	3540
catcgctccc	ttgcattctg	gcattccgtga	ctacctgggc	ctgtttgccc	ttgctgtctt	3600
tggggtagaa	gagctgagca	aggcctatga	ggatgatggt	gacgactaca	gcagcatcat	3660
ggtcaaggcg	ctggggggacc	ggctggcaga	ggcctttgca	gaagagctcc	atgaaagagt	3720
tcgccgagaa	ctgtgggcct	actgtggcag	tgagcagctg	gacgtcgcag	acctgcgcag	3780
gctgcggtac	aagggcatcc	gcccggctcc	tggctacccc	agccagcccg	accacaccga	3840
gaagctcacc	atgtggagac	tcgcagacat	cgagcagctc	acaggcatta	ggttaacaga	3900
atcattagca	atggcacctg	cttcagcagt	ctcaggcctc	tacttctcca	atttgaagtc	3960
caaataatttt	gctgtgggga	agattttccaa	ggatcagggt	gaggattatg	cattgaggaa	4020
gaacatatctt	gtggctgagg	ttgagaaatg	gcttggaccc	attttgggat	atgatacaga	4080
ctaaacttttt	ttttttttgc	ctttttttatt	cttgatgatc	ctcaaggaaa	tacaacctag	4140
ggtgccttaa	aaataacaac	aacaaaaaac	ctgtgtgcat	ctggctgaca	cttacctgct	4200
tctggttttc	gaagactatt	tagtggaacc	ttgtagagga	gcagggctct	cctgcagtgc	4260
ctggaaaaca	ggcgctgttt	ttttgggacc	ttgcgtgaag	agcagtgagc	agggttcctg	4320
tggtttccct	ggctccctctg	agatggggac	agactgaaga	cagaggctcg	ttgatttcaa	4380
agcaagtcaa	cctgcttttt	tctgttttta	cagtggaaatc	taggaggcca	cttagtcgtc	4440
tttttttccct	cttagaagaa	aagcctgaaa	ctgagttgaa	tagagaagtg	tgacctgtg	4500
acaaaatgat	actgtgaaaa	atggggcatt	ttaatctaag	tggttataac	agtggtattct	4560
gacggggaag	gtgtagctct	gttctcttcg	gaagacctcg	ttttctaaag	gctggactaa	4620
atggctgcag	aactcccttt	ggcaaaaagg	atgcgctcac	tgcttgcttg	tcagaaacac	4680
tgaagccatt	tgccccagtg	tggccaagca	gccatgcttt	ctgggcattt	tcgtctctcc	4740
ataatttcat	atttccgtac	ccctgaggaa	acaaaaagga	aatgaggaga	gaaagttact	4800

```

gttaaggggtg gttaacattht tttttgtttt gttttgtttt ggtttttttt ttttgagaca 4860
gagtctggct ctgtcgccca ggctggagtg caggggcgca atctcggctc atagcaagct 4920
ccgcctcctg ggttcatgcc attctcctgc ctacgcctcc agagtagctg ggactacagg 4980
tgccccaccac cacaccgggc taatthttttg tgtttttaca aaatacaaaa aagtagagac 5040
aggatthtcac tgtgttagcc aggatggctc tgatctcccc acctcgtgat ctgcccacct 5100
cagcctccca aaatgctggg attacaggcg tgagccaccg agcctggccg gttaacatct 5160
thtaattgtt tccaggattg agcaggthct cagctgggct ctgatatccc gtgctggagt 5220
ggacaagtgg gcagcataaa gtcactcatt tcttaccatt ttattcccct caattctcaa 5280
tatattcagt aatgaagaat ggtgccacca ctcaagcaac aagcctcaaa ctcaaccatg 5340
tcatctthtt cttggatgat tgcagttatt tcaaaaattt gcatgcaaaa tataactca 5400
tcctacttca agatgggtgg ggcaatagtc aggagaagg aacattggag tcctggthtg 5460
attcgaagga tgaagacgaa gaagcaaggg aggaacaaat gaagaacct cttgttcat 5520
gaataggaat attcaagatt ataaaggat caggtctcct aaaattgatc tatggattta 5580
ataccattht caatggaaat tccaacagat thtattgaat gaaacaagca ggtgtttata 5640
tggagtagca aaggacttaa aattaccaa tgcttctaaa tatgaaggag aggttgggga 5700
cacgcaccct atgtgatacc aagthttatt gtcaagacag tgtcatggtg cagaggtagg 5760
cattctgagc aggggaacaa aataagggcc tagaaactca cccgtgcata tgttgacct 5820
tgcaaatga cctgggtgaca tggcaagtc tgggggacag gaaggaccac tccctaagta 5880
atcccagaac aatggctatt catgtgggaa aaaaagaaat thtactthct ctaccttac 5940
ctgggtataa gttccaaata tgttaagggc thtaatacaa aaagcaaaaa ttgtcagtgt 6000
ttggatgaaa aaagccttag ggcaggaaag aatctcttga gacataaagt agtaatcata 6060
aaggacaaga tggttaagtc aattctgtta aaactcaagg cttatattaa gcaaacactt 6120
gaagtgagaa gatgatccac aacttgagaa gacatthata atacaaataa ctgatgaagg 6180
attcataatc acaaatatag agaattccta thtaaaaaaa tagaaaaata gtgaagacta 6240
cacaagagga aatagggtct thaaataaat agatgttctg tagcattggg cagggaaata 6300
tgaattagga ccacaatgag attccattht atatccataa gatttgcaa ggttgggtct 6360
gacagtacca gttgttagat ctgtagggac ttgtacaaca ttgtggatgt gtaaacaggc 6420
accactgctt taaaaaacaa ttatccctta cagacttgaa catthgcaga cgttatgatc 6480
ttgcttccaa ctcccacctg tatgtccagc aaactcttgc atgtggccac taggaggaat 6540
gtgtaagaat gttcatagtt acatatttat aatagthaat aactggaaaa agtgaaatgt 6600
atgtctgtct acaggaaaat aggtgaataa ttagatatat atattcattc tacgggatat 6660
tattcagtag tggaaatgag tgaactacag ctataacctca caataagaat gaatctcaga 6720
aaatattaag gaaaaaagca agthtgaaga gaccacatgg ggcgtactat thttattggg 6780
cccaaaaaa agcaaaaacca aagaatatgt agtctaagca tacgtataca ataaaactat 6840
gctattaaaa aaaaaaggta actgataaac caaaattgag catagtaatt acccacagaa 6900
ggaggaagtg gaagggacag gagcacatag ttagatgcca agttatgcag ctgttctggg 6960
tcctcctggg aggtttacaa gtgtttacta tatgttatta atacattata ctttataact 7020
aatagataac agthttttac atattaaata tgttctactt aaatatatta taaaaataa 7080
aggcaagtg gaatgtthta aaaaaaaaaa aaaaaaaaaa aa 7122

```

<210> 3

<211> 564

<212> DNA

<213> Homo sapiens

<400> 3

```

atggttgggt cgctaaactg catcgtcgct gtgtcccaga acatgggcat cggcaagaac 60
ggggacctgc cctggccacc gctcaggaat gaattcagat atttccagag aatgaccaca 120
acctcttcag tagaaggtaa acagaatctg gtgattatgg gtaagaagac ctggttctcc 180
attcttgaga agaatcgacc thtaagggt agaattaatt tagttctcag cagagaactc 240
aaggaaacct cacaaggagc tcattthctt tccagaagtc tagatgatgc cttaaaactt 300

```

```

actgaacaac  cagaattagc  aaataaagta  gacatggctc  ggatagttgg  tggcagttct  360
gtttataagg  aagccatgaa  tcacccaggc  catcttaaac  tatttgtgac  aaggatcatg  420
caagactttg  aaagtgcac  gttttttcca  gaaattgatt  tggagaaata  taaacttctg  480
ccagaatacc  caggtgttct  ctctgatgtc  caggaggaga  aaggcattaa  gtacaaattt  540
gaagtatatg  agaagaatga  ttaa
                                                    564

```

<210> 4

<211> 2158

<212> DNA

<213> Homo sapiens

<400> 4

```

gcgcggcata  acgacccagg  tcgcggcgcg  gcggggcctg  agcgcgtggc  cggtgccgca  60
ggagccgagc  atggagtacc  aggatgccgt  gcgcagtctc  aataccctgc  agaccaatgc  120
cggctacctg  gagcaggtga  agcgcagcgc  gggtgaccct  cagacacagt  tggaaagccat  180
ggaactgtac  ctggcacgga  gtgggctgca  ggtggaggac  ttggaccggc  tgaacatcat  240
ccacgtcact  gggacgaagg  ggaagggctc  cacctgtgcc  ttcacggaat  gtatcctccg  300
aagctatggc  ctgaagacgg  gattcttttag  ctctccccac  ctggtgcagg  ttcgggagcg  360
gatccgcctc  aatgggcagc  ccatcagtc  tgagctcttc  accaagtact  tctggcgcc  420
ctaccaccgg  ctggaggaga  ccaaggatgg  cagctgtgtc  tccatgcccc  cctacttccg  480
cttcttgaca  ctcatggcct  tccacgtctt  cctccaagag  aaggtggacc  tggcagtggt  540
ggaggtgggc  attggcgggg  cttatgactg  caccaacatc  atcaggaagc  ctgtggtgtg  600
cggagtctcc  tctcttgga  tcgaccacac  cagcctcctg  ggggatacgg  tggagaagat  660
cgcattggcag  aaagggggca  tctttaagca  aggtgtccct  gccttcactg  tgctccaacc  720
tgaaggtccc  ctggcagtg  tgagggaccg  agcccagcag  atctcatgtc  ctctatacct  780
gtgtccgatg  ctggaggccc  tcgaggaagg  ggggcccgcg  ctgaccctgg  gcctggaggg  840
ggagcaccag  cgggtccaac  ccgccttggc  cttgcagctg  gccactgct  ggctgcagcg  900
gcaggaccgc  catggtgctg  gggagccaaa  ggcattccagg  ccagggtctc  tgtggcagct  960
gcccctggca  cctgtgttcc  agcccacatc  ccacatgcgg  ctcggtcttc  ggaacacgga  1020
gtggccgggc  cggacgcagg  tgctgcggcg  cgggcccctc  acctggtacc  tggacggtgc  1080
gcacaccgcc  agcagcgcgc  aggcctgcgt  gcgctgggtc  cgccaggcgc  tgcaggggcg  1140
cgagaggccg  agcgggtggc  ccgaggttcg  agtcttgctc  ttcaatgcta  ccggggaccg  1200
ggacccggcg  gccctgctga  agctgctgca  gccctgccag  ttgactatg  ccgtcttctg  1260
ccctaacctg  acagaggtgt  catccacagg  caacgcagac  caacagaact  tcacagtgc  1320
actggaccag  gtcttgcctc  gctgcctgga  acaccagcag  cactggaacc  acctggacga  1380
agagcaggcc  agcccggacc  tctggagtgc  cccagccca  gagcccgggtg  ggtccgcctc  1440
cctgttctg  gcgccccacc  caccacacac  ctgcagtgcc  agctccctcg  tcttcagctg  1500
catttcacat  gccttgcaat  ggatcagcca  aggccgagac  cccatcttcc  agccacctag  1560
tcccccaaag  ggcctcctca  cccacctgt  ggtccacagt  ggggccagca  tactccgtga  1620
ggctgctgcc  atccatgtgc  tagtcactgg  cagcctgcac  ctggtgggtg  gtgtcctgaa  1680
gctgctggag  cccgcactgt  cccagtagcc  aaggcccggg  gttggagggtg  ggagcttccc  1740
acacctgcct  gcgttctccc  catgaactta  catactaggt  gccttttgtt  tttggctttc  1800
ctggttctgt  ctgactggc  ctaggggcca  gggctttggg  atgggaggcc  gggagaggat  1860
gtctttttta  aggtctctgt  ccttggtctc  tccttctct  tggctgagat  agcagagggg  1920
ctccccgggt  ctctcactgt  tgcagtggcc  tggccgttca  gcctgtctcc  cccaacaccc  1980
cgcttgcctc  ctggctcagg  cccagcttat  tgtgtgcgct  gcctggccag  gccctgggtc  2040
ttgccatgtg  ctgggtggta  gatttccctc  tcccagtgcc  ttctgggaag  ggagagggcc  2100
tctgcctggg  acactgcccc  acagagggtg  gctggagtga  attaaagcct  ttgttttt  2158

```

<210> 5

<211> 7720

<212> DNA

<213> Homo sapiens

<400> 5

```

taagttgaca cttctcaggt tgtcacaaga ttcaggtatg gctcactgtt gcaggacata 60
agctgggagc tectgggaat tggcttgctt gcaggcccta gagagccttc cttcttggtt 120
gattttcttc tagagatcca actgtcttct caggctcccc tgcctgcctc ctcttggggt 180
cctttcttgt ggcattgcca gattactggg cccccatttt ccctacactt actgccactc 240
atagtctgat ggttcccaca tctgcatcca acctggactc tccccctgag ctttcccctc 300
tacaaccacc ttccccgggc caagggcaca caggcacctc gacaaaacag tgttctatgt 360
ttcttcctgc ccaaacctgc ccctccctct cctttttccc atctgtggta ccaccatggg 420
ctcagagaat aaaaaaatg aaggcttctg tcattgactg gggaggagat ggagggaaga 480
gttagccagc aatcacaggt gctgtagaaa ggatacctga gttgccggga gagggggtcc 540
atgagttggg gatggaagga gagcttgccc cttcaaaca ttgaagatct gatcaaaaga 600
ttcagaacat ctgtgatttt gtggctgggt atgggtgaca cctgggctaa tgggggtggg 660
ggagttgggt gctctacaat ttatggcctt gggagatcct tgctctctat agctgactgg 720
gaggttgga gctgggctc tagcccttgc cttgatcctc cggatctcat tttcctcatc 780
tgcctaacag gacagagggg ttggaaactg atgagattag ctcaaaggat cctggcagct 840
caggctgcaa gatttttttc agacctcagt gtttgggaaa aaattgggtg ggtggagctt 900
agggactggc cttaggcctg cactgttaat tcacccctc ccactacccc atggaggcct 960
ggctgggtgct cacatacaat aattaactgc tgagtggcct tcgccaatc ccaggctcca 1020
ctctggggtc ccattcccac tccctgcctg tctcctaggc cactaaacca cagctgtccc 1080
ctggaataag gcaaggggga gtgtagagca gagcagaagc ctgagccaga cggagagcca 1140
cctcctctcc caggtatgtg aactcccca tcccccttca gaggccacac accctatggc 1200
atccccacca tgtgttaagg attttctgaa ctggaagggc cctctgtttg cctgaaggcc 1260
agagaatctt gaagtggaga ctgaggccca gaccagagtg tggcctgctc aagattaaac 1320
gacaagttag tgttcatccc cctgaactag tacctggggt ctagcccttc agtccagagc 1380
tgagttctca gctcttctag tctggggccc caaggttggg tgtgggggtc atgattgttg 1440
gtggggaggg gtcacagctg gactaagacc tgaaggtgag actaggcagg tgggaaagga 1500
gcttgagag tgatgctgct caaaaggaca ggaagagagc ctggcttcag aagcagccac 1560
agcaagagag actactgact gaacaggtgg gctccactgg gggctccgga aaggattttc 1620
tcagccccc a tccccagcac tgtgtgttg ccgcacccat gagagcctca gcaactctgaa 1680
ggtgcagggg gcaaaggcca aaagagctct ggctgaact tgggtgggtc ctactgtgtg 1740
acttggggca tggccctcat ctgtgctgaa atgattccac aaagattaaa ctggctatca 1800
tttgttgatt tcccccttct tacatttaat ccttcagga gaaagctaag cctcaagata 1860
gtttgcttct ctttccccca aggccaagga gaaggtggag tgagggtggt ggtcgggaca 1920
ggttgaacgg gaacctgtg ctctaaacag ttagggtttg tccccgcagg aactgaaccc 1980
aaaggatcac ctggtattcc ctgagagtac agatttctcc ggcgtggccc tcaagggttag 2040
tgagttagca ggtccacagg ggcattgatt gatcctggaa tgaatgaatc aacctagaga 2100
gagtgaatga acactggaat caaatagagta gcagagtaat ggattgtgga gcaggaaaga 2160
gagctgctgg gtgggaattc aattccaggc ttatatgagc cctgctgtgc agtccgcctg 2220
gagacagccc agctcaggcc ctgcctagac ccctgtcaag gaggccctgt caagaggaga 2280
ggagggggcag cacgggggca aggcaagctt gtgagcggga aaggcatgtc cactttagcg 2340
actggtatgt ggaagatgag ttagaggaga cagatggaga gaagtcatag gaaataaatt 2400
ctgagcattt taggagggcc cagacacctg gtgtccagtg gagtgaagga aacagtcgcc 2460
tcccaaaatt cagtgtctga ggtcaaagga ttgaagttct gtgatgacca aggagaagcc 2520
agctctgtgg tagggggcac aggagctccc caaggcccca gggctgtcca gctggctgtc 2580
ccctgccagc acccatgtcc tgtgacccca cccaccaag atccccatgt ttcggggaag 2640
ggcctactaa actagcttga gtgatgaggc tagaaagggg ctgggaccaa ggtttaaaaa 2700
gcaaaaacaaa ctaaaaaaaa ccacactgca gccccccaa ctaaaacatt tttataaact 2760
tttttttttt ttttgagatg gagtctcgct ctgtcaccca ggctagagtg caatggcaca 2820

```

atcttggctc	actgtaacct	ccacctcctg	gattcaagtg	attctcctgc	ctcagcctcc	2880
cacgtagctg	ggactacagg	cacacgacac	cgcacccagc	tcatttttga	tttttagtag	2940
agacagggtt	tcactatgtt	ggccaggctg	gtctcaaaact	tctgacctca	ggtgatccac	3000
ccacctcagc	cttccaaagt	gctgggatta	caggcatgag	ccaccgcgcc	cagcccattt	3060
ttgtaaactt	ttacaatgaa	gtaatttgggt	gtcaaaatct	gacctgaaaa	ttaatgtgag	3120
tttatgtata	gttttaattt	atcccactag	tgtaactgtt	tcaccccgaga	atatacactt	3180
gattattggg	tatatgaaaa	aaatatatttc	tttgaatcac	ctttgatgaa	atcctaaaaa	3240
attttaaccc	tgaaacattt	gaataaggca	ttgtggacct	atggcaaact	cctggctatt	3300
tctgcatttt	gccc aaatcc	atccttgaat	tatatcacct	gaacctcgtg	accacctgga	3360
gaaggcaatg	aggctcaagc	caggaggggg	tggtgtctaa	tcctaccttt	cattggatct	3420
gggaaaaactg	agggagatgg	gggcagggtt	ctatctgccc	caggcttccg	tccaggcccc	3480
accctcctgg	agccctgcac	acaacttaag	gccccacctc	cgcattcctt	ggtgccactg	3540
accacagctc	tttcttcagg	gacagacatg	gctcagcgga	tgacaacaca	gctgctgctc	3600
cttctagtgt	gggtggctgt	agtaggggag	gctcagacaa	ggattgcatg	ggccaggact	3660
gagcttctca	atgtctgcat	gaacgccaaag	caccacaagg	aaaagccagg	ccccgaggac	3720
aagttgcatg	agcagggtgg	ccagggggtg	atctgggggtg	gtgagggact	ggctcaggaa	3780
gaggaaacga	ggacatggaa	atgccaaacc	ccattggcac	tggtgaactg	aagtggagga	3840
gcccttcagt	ttgcattaat	atgggtgact	tatttcagag	acactgtgcc	aaatgtcgtt	3900
acaatgccaa	cagttcacct	tcttggttgt	tgagtttccg	cattacagaa	ataagggaagc	3960
aggcccaaaag	gagagcctgg	gaaatgaagt	tggagtgacc	catcctgggg	ttgcttgatt	4020
tagggattta	gactgggaat	gactcctcca	aagatctgag	ggaagaaact	gcacactgtg	4080
catagtggcc	tcttttctgc	cagccctaaa	cagctcaaga	agggagagtc	tctcacatta	4140
tgaggctgtg	tgcaaagcat	tctttttttt	ttttcctgag	acaaagtctc	catatgttgc	4200
ccaggctggg	ctcaaattcc	tggactcaag	tgatcctccc	acctcagccc	tcccaaagtg	4260
tgggattaca	gaaatgagcc	gtacgccctc	ctgaagcatc	ttggttcatg	catctcgcga	4320
aactttgggc	tgtgtctctc	gaccacattg	gacctgaggt	ctccctataa	cattttattt	4380
gctaccaccc	ctttaatatc	ctgaacatga	tgatataact	aaagaaaaag	cagaggaaaa	4440
gtaatttgta	ggccagggtg	tacggctcac	gcctgtaatc	ccaacactgt	gggatgtcga	4500
gatgggcaga	tcacttgagc	tcaggagttc	gagaccagcc	tgggcaagat	ggcaaaaccc	4560
catctctact	aaaaaataaa	aaaaattagt	caggtgtggt	ggcacatgcc	tgcatgccca	4620
gctactcagg	aggctgaggt	gggcaggcca	gttgagccca	ggaggcagag	attgtagatc	4680
gtgccactgc	actccagcct	gggcaacaga	gtgagacctt	gtcaaaagaa	agaaagaacg	4740
aaaaaaagaa	agaaaggaag	gaagggaagg	gagggaaggaa	agggaggagg	gaaagggagg	4800
gaggaaagg	agggaggcaa	gggagagaaa	cttgtaatac	gcatttcttt	ttttttttct	4860
tgagatagag	ttttgctctt	gttgcccagg	gtggatggca	gtggcacaat	ctcagctcac	4920
tgcaacctcc	acctcccagg	ttcaagtgat	tctcctgcct	cagcctcctg	agtaggcaca	4980
cgcaccaca	cccagcta	tttttgtttg	tttgtttgtt	ttgtttgttg	gtatttttag	5040
tagagatggg	ggtttcacca	tgttgccag	gctggtctcg	aactcctcac	ctcataatcc	5100
gccctcttg	gectcccaaa	gtgctgagat	tacaggtgtg	agccactgcg	cccggcctta	5160
agtgcacatt	ttattttatt	atttatttat	ttattttattg	agatggagtc	ttgctctgtt	5220
gcccaggctg	gagtgcagtg	gcacaatctc	agctcactgc	aacctccacc	tcccaggttc	5280
aagcaattct	tctgccttgg	cctccagagt	agctgggact	ataggcacct	gccaccatgc	5340
ctagctaatt	tttgtatttt	tagtagaaat	gggtttttgc	catgttggcc	aggctggtct	5400
ccattcttga	ccttaagtga	tctgtccacc	tccacctccc	aaagtgtctg	gattacagge	5460
actatgtgag	ccactgtgac	ggcccacatt	ttaatattta	gcttgtcagc	cttaagta	5520
gagattcagg	aagcttgagg	ataggcacac	aggagcatag	tttcaagttg	tcctgaattt	5580
tgacggcatc	acaagttagt	ttttaaggaa	aaagattagt	tcctaagttg	tttctcaata	5640
acttcaata	aaataacatc	cacaattgat	tggctatata	ttgttttttt	gtatcacaaa	5700
ttccacaac	agataatggg	tgaggcagct	agtcagggac	aaaacacttc	ccaagttagt	5760
gggattacag	gtgtccgcca	ccacacttgg	ctagtttttt	gtttgtttat	tttttgagat	5820
ggagtcttgc	tctgtcgccc	aggctggagt	gcagtggcat	gatctcggtc	cactgcaagc	5880

```

tccacctgcc gggttcacac cattctcctg cctcagcctc ccaagtagct gggactacag 5940
gtgccagcca ccacgcccgg ctaatttttt gtatttttag tagagacggg gtttcacat 6000
gttggccagg atggtcttga tctcttagcc tcgtgatcca cccgcctcgg cctcccaaaa 6060
tgctgggatt acaggcgtga gccaccgcac ccggcctaatt ttttatattt ttagtagaga 6120
cgggggtttca ccatggttggc caggctggtc tcaaactctt gatctcaggt gatccacctg 6180
ccttggcctc ccaaagtgtt gggattacac aagtaagcca ctgcacccag cctgggggta 6240
caatttaaat tgctttttta ccttcaaate tttgacacct cagtgaggct taatctgacc 6300
gcactattac actacaagtc cccatccgtc tctgtttaat tttgtccaa agcaaaaatc 6360
aggtgatgtg ttcattgttg taaccccagt ttctacaaaa gtacctgggt gagagtaagt 6420
aggatctcaa taaagggtga attaacaaat tttgtaatga ctgcaactcc agcaggagct 6480
cccttttggg ctccactgtt ctctgacggc cctctcccct aaagagggtc caatagcaag 6540
tattttcctg ggtgacttcc agtgggctgg ggaatcaagg actaagaggg gagacactgc 6600
atgtggaata ttctggctgt gctggctgtg ctggctgtgg actgagtcct ctgtcttccc 6660
ccatccagtg tcgaccctgg aggaagaatg cctgctgttc taccaacacc agccaggaag 6720
cccataagga tgtttctac ctatatagat tcaactggaa ccactgtgga gagatggcac 6780
ctgcctgcaa acggcatttc atccaggaca cctgcctcta cgagtgtctc cccaacttgg 6840
ggccctggat ccagcaggta tgcatggctt cctgcaggta caagacctag cggagcagct 6900
gagctttcca ggcattctct caggctgcaa cccagctcc agttctattc ggggctgagt 6960
tgctgggatt cttgaacctg agcccttctt ttgtatcaaa atcaccagg tggatcagag 7020
ctggcgcaaa gagcgggtac tgaacgtgcc cctgtgcaaa gaggactgtg agcaatggtg 7080
ggaagattgt cgcacctcct acacctgcaa gagcaactgg cacaagggtt ggaactggac 7140
ttcagggtgag ggctggggtg ggcaggaatg gagggatttg gaagtggagg tgtgtgggtg 7200
tggaacaggt atgtgacaat ttggagttgt agggctggca gacctcaaga tagttccggg 7260
cccagtggtt aaaggctctt cctcctctct acagggttta acaagtgcgc agtgggagct 7320
gcctgccaac ctttccattt ctacttcccc acaccactg ttctgtgcaa tgaaatctgg 7380
actcactcct acaaggtcag caactacagc cgaggagtg gccgctgcat ccagatgtgg 7440
ttcgacccag cccagggcaa ccccaatgag gaggtggcga ggttctatgc tcagccatg 7500
agtggggctg ggccctgggc agcctggcct ttctgtctta gcctggccct aatgctgctg 7560
tggtgtctca gctgacctcc ttttaccttc tgatacctgg aaatccctgc cctgttcagc 7620
cccacagctc ccaactattt gggttcctgt ccatggctcg gcctctgaca gccactttga 7680
ataaaccaga caccgcacat gtgtcttgag aattatttgg 7720

```

<210> 6

<211> 255

<212> PRT

<213> Homo sapiens

<400> 6

```

Met Val Trp Lys Trp Met Pro Leu Leu Leu Leu Val Cys Val Ala
  1              5              10              15

```

```

Thr Met Cys Ser Ala Gln Asp Arg Thr Asp Leu Leu Asn Val Cys Met
      20              25              30

```

```

Asp Ala Lys His His Lys Thr Lys Pro Gly Pro Glu Asp Lys Leu His
      35              40              45

```

```

Asp Gln Cys Ser Pro Trp Lys Lys Asn Ala Cys Cys Thr Ala Ser Thr
      50              55              60

```

```

Ser Gln Glu Leu His Lys Asp Thr Ser Arg Leu Tyr Asn Phe Asn Trp

```

65	70	75	80
Asp His Cys Gly Lys Met Glu Pro Ala Cys Lys Arg His Phe Ile Gln	85	90	95
Asp Thr Cys Leu Tyr Glu Cys Ser Pro Asn Leu Gly Pro Trp Ile Gln	100	105	110
Gln Val Asn Gln Thr Trp Arg Lys Glu Arg Phe Leu Asp Val Pro Leu	115	120	125
Cys Lys Glu Asp Cys Gln Arg Trp Trp Glu Asp Cys His Thr Ser His	130	135	140
Thr Cys Lys Ser Asn Trp His Arg Gly Trp Asp Trp Thr Ser Gly Val	145	150	155
Asn Lys Cys Pro Ala Gly Ala Leu Cys Arg Thr Phe Glu Ser Tyr Phe	165	170	175
Pro Thr Pro Ala Ala Leu Cys Glu Gly Leu Trp Ser His Ser Tyr Lys	180	185	190
Val Ser Asn Tyr Ser Arg Gly Ser Gly Arg Cys Ile Gln Met Trp Phe	195	200	205
Asp Ser Ala Gln Gly Asn Pro Asn Glu Glu Val Ala Arg Phe Tyr Ala	210	215	220
Ala Ala Met His Val Asn Ala Gly Glu Met Leu His Gly Thr Gly Gly	225	230	235
Leu Leu Leu Ser Leu Ala Leu Met Leu Gln Leu Trp Leu Leu Gly	245	250	255

<210> 7

<211> 817

<212> DNA

<213> Homo sapiens

<400> 7

```

cgcaggaata gatggacatg gcctggcaga tgatgcagct gctgcttctg gcttttgtga 60
ctgctgctgg gagtgcccag cccaggagtg cgcgggccag gacggacctg ctcaatgtct 120
gcatgaacgc caagcaccac aagacacagc ccagccccga ggacgagctg tatggccagt 180
gcagtccctg gaagaagaat gcctgctgca cggccagcac cagccaggag ctgcacaagg 240
acacctcccg cctgtacaac ttttaactggg atcactgtgg taagatggaa cccacctgca 300
agcgccactt tatccaggac agctgtctct gagtgctcac ccaacctggg gccctggatc 360
cggcaggtca accagagctg gcgcaaagag cgcattctga acgtgcccct gtgcaaagag 420
gactgtgagc gctggtggga ggactgtcgc acctcctaca cctgcaaaag caactggcac 480
aaaggctgga attggacctc agggattaat gagtgtccgg ccggggccct ctgcagcacc 540

```

```

tttgagtcct acttccccac tccagccgcc ctttgtgaag gcctctggag ccactccttc 600
aaggtcagca actatagtcg agggagcggc cgctgcatcc agatgtgggt tgactcagcc 660
cagggcaacc ccaatgagga ggtggccaag ttctatgctg cggccatgaa tgctggggcc 720
ccgtctcgtg ggattattga ttctgatcc aagaagggtc ctctgggggt cttccaacaa 780
cctattctaa tagacaaatc cacatgaaaa aaaaaaa 817

```

<210> 8

<211> 1669

<212> DNA

<213> Homo sapiens

<400> 8

```

gctaggcagc ttcgaaccag tgcaatgacg atgccagtea acggggccca caaggatgct 60
gacctgtggt cctcacatga caagatgctg gcacaacccc tcaaagacag tgatgttgag 120
gtttacaaca tcattaagaa ggagagtaac cggcagaggg ttggattgga gctgattgcc 180
tcggagaatt tcgccagccg agcagttttg gaggccctag gctcttgctt aaataacaaa 240
tactctgagg ggtaccgggg ccagagatac tatggcggga ctgagtttat tgatgaactg 300
gagaccctct gtcagaagcg agccctgcag gcctataagc tggaccaca gtgctggggg 360
gtcaacgtcc agccctactc aggcctccct gcaaaccttg ctgtgtacac tgccctgggtg 420
gaaccccatg ggcgcacatc gggcctggac cttccggatg ggggccacct gacccatggg 480
ttcatgacag acaagaagaa aatctctgcc acgtccatct tctttgaatc tatgccctac 540
aaggtgaacc cagatactgg ctacatcaac tatgaccagc tggaggagaa cgcacgcctc 600
ttccacccga agctgatcat cgcaggaacc agctgctact cccgaaacct ggaatatgcc 660
cggctacgga agattgcaga tgagaacggg gcgtatctca tggcggacat ggctcacatc 720
agcgggctgg tggcggctgg cgtggtgccc tccccatttg aacactgcca tgtggtgacc 780
accaccactc acaagaccct gcgaggctgc cgagctggca tgatcttcta caggaaagga 840
gtgaaaagtg tggatcccaa gactggcaaa gagattctgt acaacctgga gtctcttatc 900
aattctgctg tgttccctgg cctgcaggga ggtccccaca accacgccat tgctgggggt 960
gctgtggcac tgaagcaagc tatgactctg gaatttaaag tttatcaaca ccagggtggtg 1020
gccaaactga gggctctgtc tgaggccctg acggagctgg gctacaaaat agtcacaggt 1080
ggttctgaca accatttgat ccttgtggat ctccgttcca aaggcacaga tgggtggaagg 1140
gctgagaagg tgctagaagc ctgttctatt gcctgcaaca agaacacctg tccaggtgac 1200
agaagcgctc tgcggcccag tggactgcgg ctggggaccc cagcactgac gtcccgtgga 1260
cttttgga aaagacttcca aaaagtagcc cactttattc acagagggat agagctgacc 1320
ctgcagatcc agagcgacac tgggtgcaga gccaccctga aagagttcaa ggagagactg 1380
gcaggggata agtaccaggc ggccgtgcag gctctccggg aggaggttga gagcttcgcc 1440
tctctcttcc ctctgcctgg cctgcctgac ttctaaagga gcggggccac tctggacca 1500
cctggcgcca cagaggaagc tgcctgccgg agaccccccac ctgagagatg gatgagctgc 1560
tccaaaggga actgttgaca ctcgggccct ttgaggggtt ttcttttgga cttttttcat 1620
gttttcttca caaatcaaaa tttgtttaag tctcattgtt agtaattct 1669

```

<210> 9

<211> 3112

<212> DNA

<213> Homo sapiens

<400> 9

```

gtggaacctc gatattggtg gtgtccatcg tgggcagcgg actaataaag gccatggcgc 60
cagcagaaat cctgaacggg aaggagatct ccgcgcaaat aagggcgaga ctgaaaaatc 120
aagtcactca gttgaaggag caagtacctg gtttcacacc acgcctggca atattacagg 180
ttggcaacag agatgattcc aatctttata taaatgtgaa gctgaaggct gctgaagaga 240

```

```

ttgggatcaa agccactcac attaagttac caagaacaac cacagaatct gaggtgatga 300
agtacattac atctttgaat gaagactcta ctgtacatgg gttcttagtg cagctacctt 360
tagattcaga gaattccatt aacactgaag aagtgatcaa tgctattgca cccgagaagg 420
atgtggatgg attgactagc atcaatgctg ggagacttgc tagagggtgac ctcaatgact 480
gtttcattcc ttgtacgcct aaggggatgct tggaaactcat caaagagaca ggggtgccga 540
ttgccggaag gcatgctgtg gtggttgggc gcagtaaaat agttggggcc ccgatgcatg 600
acttgcttct gtggaacaat gccacagtga ccacctgcca ctccaagact gcccatctgg 660
atgaggaggt aaataaagggt gacatcctgg tgggtgcaac tggtcagcct gaaatggtta 720
aaggggagtg gatcaaacct ggggcaatag tcatcgactg tggaaatcaat tatgtcccag 780
atgataaaaa accaaatggg agaaaagggtg tgggtgatgt ggcatagac gaggccaaag 840
agaggggcag cttcatcact cctgttctctg gcggcgtagg gcccatgaca gttgcaatgc 900
tcatgcagag cacagtagag agtgccaagc gtttctctgga gaaatttaag ccaggaaagt 960
ggatgattca gtataacaac cttaacctca agacacctgt tccaagtgc attgatatat 1020
cacgatcttg taaaccgaag cccattggta agctggctcg agaaattggt ctgctgtctg 1080
aagaggtaga attatatggt gaaacaaagg ccaaagtctt gctgtcagca ctagaacgcc 1140
tgaagcaccg gcctgatggg aaatacgtgg tggtgactgg aataactcca acacccttg 1200
gagaagggaa aagcacaact acaatcgggc tagtgcaagc ccttgggtgcc catctctacc 1260
agaatgtctt tgcgtgtgtg cgacagcctt ctgagggcc cacttttga ataaaagggt 1320
gcgctgcagg aggcggctac tcccaggtea ttcttatgga agagttaat ctccacctca 1380
caggtgacat ccatgccatc actgcagcta ataacctcgt tgctgcggcc attgatgctc 1440
ggatatttca tgaactgacc cagacagaca aggtctctct taatcgtttg gtgccatcag 1500
taaattggagt gagaagggtt tctgacatcc aaatccgaag gttaaagaga ctaggcattg 1560
aaaagactga cctaccaca ctgacagatg aagagataaa cagatttgca agattggaca 1620
ttgatccaga aaccataact tggcaaagag tgttggtatc caatgataga ttcctgagga 1680
agatcacgat tggacaggct ccaacggaga agggtcacac acggacggcc cagtttgata 1740
tctctgtggc cagtgaatt atggctgtcc tggctctcac cacttctcta gaagacatga 1800
gagagagact gggcaaaatg gtggtggcat ccagtaagaa aggagagccc gtcagtggcc 1860
aagatctggg ggtgagtggt gcactgacag tgcttatgaa ggacgcaatc aagcccaatc 1920
tcatgcagac actggagggc actccagtgt ttgtccatgc tggcccgttt gccaacatcg 1980
cacatggcaa tctctccatc attgcagacc ggatcgcaat caagcttggt ggcccagaag 2040
ggtttgtagt gacggaagca ggatttgag cagacattgg aatggaaaag ttttttaaca 2100
tcaaattgcc gtattccggc ctctgcccc acgtgggtgg gcttggtgcc actgtcaggg 2160
ctctcaagat gcacgggggc ggccccacgg tcaactgctg actgcctctt cccaaggctt 2220
acatacagga gaacctggag ctggttgaaa aaggcttcag taacttgaag aaacaaattg 2280
aaaatgccag aatgtttgga attccagtag tagtggccgt gaatgcattc aagacggata 2340
cagagtctga gctggacctc atcagccgcc ttccagaga acatggggct tttgatgccg 2400
tgaagtgcac tcaactgggc gaagggggca aggtggcctt agccctggct caggccgtcc 2460
agagagcagc acaagcacc agcagcttcc agctccttta tgacctcaag ctcccagttg 2520
aggataaaat caggatcatt gcacagaaga tctatggagc agatgacatt gaattacttc 2580
ccgaagctca acacaaagct gaagtctaca cgaagcagg ctttggaat ctccccatct 2640
gcatggctaa aacacacttg tctttgtctc acaaccaga gcaaaaagg gtccctacag 2700
gcttcattct gcccatctgc gacatccgcg ccagcgttgg ggctggtttt ctgtaccctt 2760
tagtaggaac gatgagcaca atgcctggac tccccaccg gccctgtttt tatgatattg 2820
atttgacccc tgaaacagaa caggtgaatg gattattcta aacagatcac catccatctt 2880
caagaagcta ctttgaaagt ctggccagt tctattcagg cccactggga gtttaggaagt 2940
ataagtaagc caagagaagt cagccctgc ccagaagatc tgaaactaat agtaggaggt 3000
tccccagaag tcattttcag ccttaattct catcatgtat aaattaacat aaatcatgca 3060
tgtctgttta ctttagtgac gttccacaga ataaaaggaa acaagtttgc ca 3112

```

<210> 10

<211> 1792

<212> DNA

<213> Homo sapiens

<400> 10

```

cgcagcccag actcagactg ggggaagcaaa caggggctgg acaggccagg agagcctgtc 60
ggacagtgat cctgagatgt gggagttgct gcagagggag aaggacaggc agtgtcgtgg 120
cctggagctc attgcctcag agaacttctg cagccgagct gcgctggagg ccctgggggtc 180
ctgtctgaac aacaagtact cggaggggtta tcctggcaag agatactatg ggggagcaga 240
gggtgtggat gaaattgagc tgctgtgcca gcgcccggcc ttggaagcct ttgacctgga 300
tcctgcacag tggggagtca atgtccagcc ctactccggg tccccagcca acctggccgt 360
ctacacagcc cttctgcaac ctacagaccg gatcatgggg ctggacctgc ccgatggggg 420
ccatctcacc cacggctaca tgtctgacgt caagcggata tcagccacgt ccattctctt 480
cgagtctatg ccctataagc tcaaccccaa aactggcctc attgactaca accagctggc 540
actgactgct cgacttttcc ggccacggct catcatagct ggcaccagcg cctatgtctg 600
cctcattgac tacgcccga tgagagaggt gtgtgatgaa gtcaaagcac acctgtctggc 660
agacatggcc cacatcagtg gcctgggtggc tgccaaggtg attccctcgc ctttcaagca 720
cgcgagacatc gtcaccacca ctactcacia gactcttcga ggggcccaggc cagggctcat 780
cttctaccgg aaaggggtga aggtgtgga cccaagact ggccgggaga tcctttacac 840
atltgaggac cgaatcaact ttgccgtgtt cccatccctt cagggggggcc cccacaatca 900
tgccattgct gcagtagctg tggccctaaa gcaggcctgc acccccatgt tccgggagta 960
ctccctgcag gttctgaaga atgctcgggc catggcagat gccctgctag agcagggcta 1020
ctcactggta tcaggtggta ctgacaacca cctggtgctg gtggacctgc ggcccaaggg 1080
cctggatgga gctcgggctg agcgggtgct agagcttgta tccatcactg ccaacaagaa 1140
cacctgtcct ggagaccgaa gtgccatcac accgggcggc ctgcggcttg gggcccagc 1200
cttaacttct cgacagttcc gtgaggatga cttccggaga gttgtggact ttatagatga 1260
aggggtcaac attggcttag aggtgaagag caagactgcc aagctccagg atttcaaatc 1320
cttctgctt aaggactcag aaacaagtca gcgtctggcc aacctcaggc aacgggtgga 1380
gcagtttgcc agggccttcc ccatgcctgg ttttgatgag cattgaaggc acctgggaaa 1440
tgaggcccac agactcaaag ttactctcct tccccctacc tggggccagt aaatagaaaag 1500
cctttctatt ttttggtgcg ggaggggaaga cctctcactt agggcaagag ccaggtatag 1560
tctcccttcc cagaatttgt aactgagaag atcttttctt tttccttttt ttggttaacaa 1620
gacttagaag gagggcccag gcactttctg tttgaacccc tgtcatgac acagtgtcag 1680
agacgcgtcc tctttcttgg ggaagttaga gagtgcctt cagagccagt agcaggcagg 1740
gggtgggtagg caccctcctt cctgttttta tctaataaaa tgctaacctg ca 1792

```

<210> 11

<211> 18596

<212> DNA

<213> Homo sapiens

<400> 11

```

cctgtagtcc cagctacgag agaggctgag gcagcagaat tacttgaacc caggaggcgg 60
agggttcagt gagccgagat cgcgccactg cactccagcc tgggtgagag agcgagactc 120
tgtctcaaaa aaaaaaaaaa aagaccgcca gggctcaaac aaaaaacctc ggaaaagccc 180
tggcgggtctt tttttttttt tttttttttt ttttttgga cagtcttgct ctgtcgccca 240
ggctggagta caatggctcg atcttggtc actgcaacct ctgcctcca gggtcaagca 300
attcttctgc ctacgcctcc caagtagcca ccacgcccag ctaatttttg tacttttagt 360
agagacgggg gtttcaccat gttgtccagg ctggctctga actcctgacc tcaggtgatc 420
caccgcctc ggccccccaa agtactagga ttacaggcgt gagccaccgc gtccagcgcc 480
ctggcgggtt ttaatcaagt agaaaagctg cattatacca cttgcttcgg ttgcttcagt 540
gagaacgaag aaatggaaat gcaaatccct tattagttgt aggaaacaga tctcaaacag 600

```

```

cagttttggt gacaagaccg caggaaaacg tgggaactgt gctgctggct tagagaaggc 660
gcggtcgacc agacgggtcc caaaggggcg agtccttccc agccaccgca cctgcatcca 720
ggttcccggg tttcctaaga ctctcagctg tggccctggg ctccgttctg tgccacaccc 780
gtggctcctg cgtttccccc tggcgacgcg tctctagagc gggggccgcc gcgaccccg 840
cgagcaggaa gaggcggagc gcgggacggc cgcgggaaaa ggcgcgcgga aggggtcctg 900
ccaccgcgcc acttgccctg cctccgtccc gccgcgccac ttggcctgcc tccgtcccg 960
cgcgccactt cgctgcctc cgtcccccgc ccgcgcgcc atgcctgtgg ccggtcgga 1020
gctgccgcgc cgcccttgc ccccgccgc acaggagcgg gacgccgagc cgcgtccgc 1080
gcacggggag ctgcagtacc tggggcagat ccaacacatc ctccgtgcg gcgtcaggaa 1140
ggacgaccgc acgggcaccg gcaccctgtc ggtattcggc atgcaggcgc gctacagcct 1200
gagaggtgac gccgcggggc cctgcgggac ggggtggcggg aaggaggag gcgcggctg 1260
ggagagcgct cgggagctgc cggcgctgc ggaccccggt tagtcctaac ctcaatcctg 1320
ccagggaggg gacgcctcgt cctcctcgcc ttacagagc cgaaacggag ggtccatta 1380
gggacgtgac tggcgcgggc aacacacaca gcagcgacag ccgggaggta agccgcgtcc 1440
cagcggctcc gcggccgggc tcgcagtgc cccagtgatg ccgtggcccc cgaggcgggc 1500
gtcatcgggc agcgtttgcc cagtgcctgga gggttaggga gagctgcctg ggcttgaccg 1560
cgcgcgggtc tcaaagtcct ggctttggcc cctcctcgt tttccctgt ggaccattcc 1620
gcttcgcagc gttttcaaaa actggagcga aagtgatgtg ggcggggcaa aggcggcggg 1680
aagaggacag cactgaagct ggcgcgggaa cttggtttcc tgggtggcctc ccattccaatc 1740
cccacgaacc agctttcctc ttaaaccttg aaaagagaaa ttcgggagtt cgagttctta 1800
gtcgtccttt cctctttcct ttccgacagg agcaccaccg gcaaaaaatg tctcgcgggt 1860
cattggcgcc aggttttcag gggacagtgg ggcgggcgcg ggtgggcaca ggacgttagg 1920
cagccgttgg cctccctaa ggccacaccg tctgcccgtc ctggatcctg cgccagctgc 1980
cggggggagg ggactcgaag gtgtgtgagc caggggctga ccttgaccgc tcagataaat 2040
ggagcgcagc cttgacacag ggttgagggt ggttttgaat ggggaaacc attcgtggtg 2100
aagcagattc actgtagcta gcggaaaagc cctccggccc acggaccat acgagacga 2160
atacatagca gctgctgtgg ctgattggcg tgggacagcg tggggagttt tgtctgagga 2220
gagggatcca cttttctgca gctccaagcc caggggcctt tgatgagcca tagacctcat 2280
ttttaaccca cctttctgct tagacattga gcaagttact tctcatatag ctccctata 2340
tgtaaaaaat ggagaaaaata atgcttagta ggcaattctg ataaaagcag gtgcttgcaa 2400
aaatctctct gttgtctgaa tataaactgt accacaagcg agtgcggtg aacgaggact 2460
gcatttaaa atagttttt acactttcat ttctctgtgg ctcgacact ctgatgcctc 2520
cctttttgtt cctgggacac atgcttggtg ttgtcttcac acctttgtga caggattagc 2580
actagtgggc agtggtgat agctcctcct ccttttgcc acatgttcat cctgcccctc 2640
gccaccatct cactgtgtgg aattcctgtg tccactggtc accggggcac agaagtgtg 2700
tctcagcctg aatcgggcca ctgatgggac ttgcagcctg ggagctccac cgtgatctct 2760
ggccacttt gcgggagctc aggtttctg gatgtccag gcctcacgtc ccagggcagt 2820
tttcttcct gaagaaagt ggatggcatg atctgtctc ccatcttgaa accgtatggc 2880
aaattgtttt tcagatgaat tccctctgct gacaaccaa cgtgtgttct ggaagggtgt 2940
tttgaggag ttgctgtggt ttatcaaggt aaagaagtcg ctgctattag aagtcagtag 3000
tctgttctca acacagcagc cagtgaatc ctttcaaaac tcaaagcagc cagggtgtgg 3060
ggctcagcc tgtaatccca ccgctttggg aggtgtgagc agatcacctg aggttaggaa 3120
tttgggacca gcctggccaa catggcgaca cccagtcctc tactaataac acaaaaaatt 3180
agccaggtgt gctggtgcat gtctgtaatc ccagctactc aggaggctga ggcatgagaa 3240
ttgctcacga ggcggagggt gtagttagct gagatcgtgg cactgtactc cagcctggcg 3300
acagagggag aaccatgtc aaaaacaaaa aaagacacca ccaaaggta aagcatatca 3360
ttcctcacc tcaagccctt agtggctcca ttctactcag taagagccac ggtccttatg 3420
gtgtccgttt ttcagctctg accttagctg ctgctctctg caccaccctg ctgttcttgt 3480
gagtttttga gcacaccggg acatccccac tccctggaac cttcttcccc cacacttggc 3540
ttcttcttt gagtctctac tccactcggg caagccttcc tagacctcct gatttaaaac 3600
tgtgactct ccccaacctc cttggtgttt ctccgtagac gaacatcacc atctgatgta 3660

```

tgtagcctt	tcccttcccc	tgtagaagg	gggacagcag	gtagtaaaag	tgaaatgtgc	3720
tgtaagcttt	atgagggcag	aggatttgtt	tctcgtgttc	actgttgtat	cgccagggcc	3780
tcaaacacag	cctgccacat	agtaggagtc	aacatatatt	gatcactaaa	tgtagatacc	3840
acctgtgttc	ccatgttcat	ataaattcta	gaagagtctc	ttcagtaaca	aggtgaaccc	3900
cttccagagg	gctgagtagg	tacctcaggc	cggggccaga	gtgctgtgaa	gacagcagca	3960
gcccagacca	agcttctctg	tggtccgtgt	cctgggtctag	aaccagcgat	gttctttctg	4020
accagtgtctt	tttggaaagg	ggctgaggtc	tgggtctcagg	tctggggccat	actagaagct	4080
gggatccctt	ctatagagca	cttgggtatg	cttgtatggt	cttggggcaa	gccagaccca	4140
agccctctta	tcccatctta	gaaagggctt	caatttggat	ccagccccag	gtctgcctta	4200
gctctgtatt	cttgggggtat	tttgttctgt	attggcctat	cttgactaac	aatgagcctt	4260
ggatttgaaa	catatcatca	gaaacctcag	aagacaacat	tcttaaactg	gctagagcct	4320
ggtctgaatg	gatgaaaagg	agagactttt	gaagcaatat	gtaaaagatt	gagaaatgat	4380
ttgttggaag	tttctcaatt	ggagaaattt	ctttgatttg	ttggaaattt	ctttgattct	4440
ttctcaatca	aagaaaatcg	ggacaaactc	aacaatagaa	agggaggaag	caagatactc	4500
agaaataaaa	tgcattcccc	tggttcaact	taatgcttca	attcaggatt	ctaaggaatc	4560
cttgccagga	atgtcagact	caccttgata	gttgaggtta	ctccattggg	gactcgatca	4620
aatacaggag	ttgaggcacc	tgcactgtaa	aatactgatt	agtctgatca	ttaggaatat	4680
cctgtatgcc	aggtagaaga	tacattgaac	agattgcatg	taggcattaa	attcattttg	4740
gggtattaca	tatagacaac	acatttcatt	aagaaacata	aaactgtcag	atcgggtggaa	4800
tacttaaaag	cacttgaggg	tgtttagcct	aaaaagctta	gttgagggga	atggaagaaa	4860
agatctggga	gggtgggttcc	aaagaaggga	tcagactatc	ctaaagccct	caggaatctg	4920
ggctgggacc	acctacttaa	agataggatg	ggcagctggg	tggtgtggct	cacgcctgta	4980
atcccagcac	ttcgggaggc	cgaagcgggc	ggatcacctg	aggtcaggag	ttcgaggcca	5040
gcctgaccaa	catggagaaa	cgctgtctct	actaaaaata	caaaattagc	tggtgttagt	5100
ggcgcatgcc	tgtaatccca	gctactcggg	aggctgaggc	aggggaatcg	cttgaacctg	5160
ggaggtggag	ggtgccgtga	gccacgatcg	cgccattgca	ctccagcctg	ggcaacaaga	5220
gcgaaactct	caaaaaacaa	aaaaaaggat	gggttccata	tggtgtgtgt	caagtgccca	5280
cctcctagca	agtcagcagg	ggccagaggc	ccttgtaagt	ggtgtctcgg	ggggatcaac	5340
tgagatggct	taagatttac	ctggatgcct	gctctgctct	ccccatctct	tccagggatc	5400
cacaaatgct	aaagagctgt	cttccaaggg	agtgaataatc	tggtgtgcca	atgggatccc	5460
agactttttg	gacagcctgg	gattctccac	cagagaagaa	ggggacttgg	gcccagttta	5520
tggtctccag	tggaggcatt	ttggggcaga	atacagagat	atggaatcag	gtgaggagat	5580
agaacaatgc	cttccatttc	cgggtgccct	tccatagcac	tggttgctcc	gttgttttag	5640
ataaggtctg	ggggatgagt	caatgtcaca	ggagctgatg	tatagctttg	accttgtgag	5700
gggtgggtgcc	agggtgaagc	cacaattaac	gcctactgaa	ggccgtttca	catctttttt	5760
tttttttttt	ttttaattat	tatactttaa	gttttagggg	acatgtgcac	aatgtgcagg	5820
ttagttacat	atgtatacat	gtgccatgct	ggtgcgctgc	accactaact	cacctcttag	5880
catcagggtat	atctcccaat	gctatccctc	ccccctcctc	ccacccacac	acatccccag	5940
agtgtgatgt	tcccccttct	gtgtccatat	gttctcgttg	ttcgattccc	actatgagtg	6000
agaatatgcg	gtgttttggt	ttttgttctt	gcgatagttt	actgagaatg	atgatttcca	6060
tttcaccacg	tccctacaga	ggacatgaac	tcatcatttt	ttatggctgc	atagatttcc	6120
atgggtgata	tgtgccacat	tttcttaatc	cagtctatca	tggtggacat	ttgggttggt	6180
tccaagtctt	tgcctattgt	gaatagtgcc	acaataaaca	tacgtgtgca	tgtgtcttta	6240
tagcagcatg	atttaatatg	cctttgggta	tataccaggt	aatgggatgg	ctgggtcaaa	6300
tggtatttct	agttctagat	ccccgaggaa	tcgccacact	gacttccaca	atggttgaac	6360
tagtttacag	tcccaccaac	agtgtcaaag	tgctctatct	ctccacatcc	tctccagcac	6420
ctgttggttc	ctgacttttt	aatgattgcc	attctaactg	gtgtgagatg	gtatctcatt	6480
gtgggtttga	tttgcggttc	tctgatggcc	agtgatgggt	agcatttttt	catgtgtttt	6540
ttggctgcat	aaatgtcttc	ttttgagaag	tgtctgttca	tgtccttcgc	ccactttttg	6600
atgggggtgt	ttttttctta	taaatttgtt	tgagttcatt	gtagattctg	gatattagcc	6660
ctttgtcaga	tgagtagggt	gcaaaaatgt	tctcccatct	tggtgggtgc	ctgttcactc	6720

```

tgatggtagt ttcttttgcg gtgcagaagc tctttagttt aattagatcc catttgtcaa 6780
ttttggcttt tggtgccatt gcttttggca taggcatgaa gtccttgccc atgcctatgt 6840
cctgaatggg aatgcctagg ttttcttcta gggtttttat ggtttttaggt ctaacgttta 6900
agtctttaat ccactctgaa ttgatttttg tataagggtg aaggaaggga tccagtttca 6960
gctttttaca tatggctagc cagttttccc agcaccattt attacatagg gaatcctttc 7020
cccattgctt gtttttctca ggtttgtcaa agatcagata gttgtagata tgcggcggtta 7080
tttctgaggg ctctgttctg ttccattgat ctatgtgtct gttttggtac cagtaccata 7140
ctgttttggg tactgtagcc ttgtagtata gtttgaagtc aggtagcgtg atgcctccag 7200
ctttgttctt ttggcttagg attgacttgg cgatgcgggc tcttttttgg ttccatatga 7260
actttaaggt agttttttcc aattctgtga agaaagtcat tggtagcttg atggggatgg 7320
cattgaatct ataaattacc ttgggcagta tggccatttt cacgatattg attcttctca 7380
cccatgagca tgggaatggc ttccatttct ttgtatcttc ttttatttca ttgagcagtg 7440
gtttgtagtt ctcttgaag aggtccttca catcctttt aagggtggatt cctaggtatt 7500
ttattctctt tgaagcaatt gtgagtggaa gttcactcat gatttggctc tctgtttgtc 7560
tgttattggg gtataagaat gcttgtgatt tttgcagatt gattttatat cctgagactt 7620
tgctgaagct gcttatcagc ttaaggagat tttgggctga gacaatgggg ttttctagat 7680
atacaatcat gtcgtctgca aacaggggaca atttgacttc ctcttttctt aattgaatac 7740
cctttatttc ctctctctgc ctaattgccc tggccagaac ttccaacact atgttgaata 7800
ggagtgggtg gagagggcat ccctgtcttg tgccagtttt caaaggggaat gcttccagtt 7860
tttgccatt cactatgata ttggctgtgg ctttgtcata gatagctctt attattttga 7920
aatatgttcc atcaatacct aatttattga gagtttttag catgatgtgt tgttgaattt 7980
tgtcaaaggc tttttctgca tctattgaga taatcatgtg gtttttgtct ttggatctgt 8040
ttatatgctg gattacattt attgatttgc gtattattgaa ccagccttgc atcctaggga 8100
tgaagccac atgatcatgg tggataagct ttttgatgtg ctgctggatt cggtttgcca 8160
gtattttatt gaggattttt gcatcaatgt tcatcaagga tattggtcta aaattctctt 8220
ttttggtgtg tctctgccc gctttggtat caggatgatg ttggcttcat aaaatgagtt 8280
agggaggatt ccctcttttt ctattgattg gaatagtttc agaaggaatg gtaccagttc 8340
ctctttgtac ctctggagaa ttcggctgtg aatccatctg gtccctggact ctctttgggt 8400
ggtaagctat tgattattgc cacaatttca gctcctgtta ttggtctatt cagagattca 8460
acttcttctt ggtttagtct tgggagagtg tatgtgtcaa ggaatttatc catttcttct 8520
agattttcta gtttatttgc gttagaggtgt ttgtagtaat ctctgatggg agtttgtatt 8580
tctgtgggat cggtggtgat atccccctta tcatttttta ttgctcttat ttgattcttc 8640
tctttttctt tattagtctt gctagcggtc tataaaattt gttgatcctt tcaaaaaacc 8700
agcttcttga ttcatataat ttttgaaggg ttttttgtgt ctctatttcc ttcagttctg 8760
ctctgatttt agttatttct tgccttctgc tagcttttga atatgtttgc tcttgctttt 8820
ctagtctttt taattgtgat gttaggggtgt caattttgga tcttctctgc tttctcttgt 8880
gggcatttag tgctataaat ttccctctac aactgcttt gaatgtgtcc cagaggttct 8940
ggtatgttgt gtctttgttc ttgttgggtt caaagaacat ctttatttct gccttcat 9000
cgttatgtac ccagtagtca ttcaggagca ggttgttcag tttccatgta gttgagcagt 9060
tttgagttag attcttaatc ctgagttcta gtttgattgc actgtggtct gagagatagt 9120
ttgttataat ttctgttctt ttacatttgc tgaggagagc tttacttcca actatgtggg 9180
cggttttggg atagggtggg tgtggtgctg aaaaaaatgt atattctgtt gatttgggat 9240
ggagtctctg agatgtctat taggtctgct tgggtcagag ctgagttcaa ttcctgggta 9300
tccttgttga ctttctgtct cgttgatctg tgtactgttg acagtgggtg ttaaagtctc 9360
ccattattaa tgtgtggagt ctaagtctct ttgtaggtca ctcatgatg tggcacttac 9420
tgggcgcttg gcactttcca tactgtgtca tcggcagata gctgcatggg tgggttctgt 9480
gctggggaat ggggaagttca tcggtgggac aaggacaaaa tgccccatt gctttgttgt 9540
ggctttaatc tccctttcga ggctgagcca cagcgtgctg taggtggcgc tgcgtggaag 9600
cgcagtacca gggtcacact ccactcccag ctctgcagag gtggagaaag aatgaaacat 9660
ctcactcctg gacttccact ttctgtcac tgttgggtgc acctcttact ggatgtcaca 9720
gagcccagcc cctcccacct gtgcctagga aaagcagatg ccacctgga atgtgggggt 9780

```

```

tgtgtgtgca atttactagc tgggcagaga ccagcaacct ggagagcagg tgtctcgtct 9840
aaggggacag tcacatttca cctccagcca cctggaggaa tttgggcctg gtgatgtcag 9900
aattcttcaa taaaagccta aaatctatat ttatgtgcg gtcatgagat ctgttaaagt 9960
ttagcaactt caggaagttt aaaaatgctg tgtggacctt gaataggcaa gttcttaaag 10020
gcagaaagtg gaatgctagt ttccagggac tggggaacag ggaggaatgg ggagttcatg 10080
tttaatgggc acagaggttt tgtagggat gacgaaaaag ttcgggagat ggtgatggtg 10140
atggagatgg tgatggtgat ggagatggtg atggtgatgg tgatggtgat ggtgatggtg 10200
gatggtgatg gtgatggtga tggagatggt gatggtgatg gtgatggaga tgggatggtg 10260
gatggtgatg gtgatggaga tgggatggtg gatggagatg gtgatggtga tgggatggtg 10320
gatggtgatg gtgatggtga tgggatggtg gatggtgatg gtgatggaga tgggatggtg 10380
gatggtgatg gttgcctaac atcaggaacg tgcttaatgc ttctgaattg cacacaaaaa 10440
tggcaagttt aatattatgt gtactttatc acaatgaaaa aagctgctgc gtgggccaaag 10500
ttacttgtgc aggtaatgtt ctgcagggtg ttgcctgcac ctgagttgta ggggtgtccgt 10560
aggatgtgag gccagtcctc gggtctaatg atgctttaa tctgcctag tattcaatta 10620
tttcttgtcg cttaaaaggc ctaataaaat tatggtctta gtttacagtg gtatgaatgc 10680
ttagctgttg gatttttagta ggaaagttcg tccctttttg tttttaattt tgttttacag 10740
attcacagga attttttttt tttttttttt tttttttttt taatgcacag aaagtttccc 10800
tggactctct acccagtttc cccagtata atactttggg taacatcctg tataattca 10860
cattggtgca ttctcagag ttgtcagatt ttgctagttt tacgtgcact tgtgtatgtg 10920
tgtatttgca attttagcac gtgtagactc ttgtaaccac tacaatcaag ttacagaact 10980
acactaccaa ggttcatctt tttaaaatct ttgatgttac cttttttgga acagtgacca 11040
tgagaggact ttctcccaa aattttgaaa actactgaac cagaatatag tctgacacta 11100
ataggtagaa atttaaccaa aggagattat gaagctctgc acttgagtta acaaaatcac 11160
ttctcagctt ccagttccat ctgagaagg agggaaaagg attaaaaatc cagagaccag 11220
aaaatgggag caaagtacaa ggtggtgtaa tcattacaga ggtttcctga tgtttccaag 11280
tcagtcgtgt gttgagctgc taaactctaa agtaatttta ggtggaatgt tggaaacatg 11340
ctgctgaggt gatagaaagg aatccatggt cctctgttag ttggaagta tatggaatac 11400
tatattctac ataagataca atactctctg tgagacaagg ataaagtaga ttttgtcagt 11460
gaaattgtga caagaatcgc tgatgggttt agagcctaag tttgagagg gactggaag 11520
aaattaagat tgttgagatt ggaaagggtt agctatgggg gaacaggagg aggtgactcc 11580
atgacagacc aatatattcaa aggactgtgt agaagaggaa aaagactttg ttagggctcc 11640
agaggacaga gccaggagtc agacagggcc ttgaactcaa cccaccgaga tctgcaaaact 11700
ttgcaggatg caccagatgt cttgtagcca tgggtcaagg ggggaccctg ggtaagagac 11760
tgtaatagat gacctctaag gccatctcat attaatgtat gtacctgtcc 11820
tctctttttg acaattctac agattattca ggacaggagg ttgaccaact gcaaagagt 11880
attgacacca tcaaaaccaa ccctgacgac agaagaatca tcatgtgcgc ttggaatcca 11940
agaggttgaa agaaccctgt cgtcttcatt tatactaacc atactcttag agggaagcaa 12000
tctgggtttg tgcagaggca ctgaggagg caggaccctg ggcaacttcc cccagccaca 12060
tgggtgtgtg acgttgggca agtcacattt tgctgcactt tcaccttcag atcatgaggt 12120
tgggcccaga ggattttttt tttttttttt ttttttgaga cagagttttg ctctgttgcc 12180
caggctggaa tgcaacggcg tgatcttggc tcaactgtaac ctctgcctcc tgggttcgag 12240
tgattctcct gcctcagcct ccaagtagct gggattacag catgtgccac catgcctggc 12300
taattttgta tttttagtag agacgggttc acatgttggg caggctgggtc ttgactcctg 12360
accctcagat gatctgcctt gcctcagcct cccaaccgag tgatcttaag ttgtgtatta 12420
tactcattct tacacaaaaa gggctttaaa tgcctagaaa ctacatgaag atgttaacat 12480
tttaaatgga agcagatgaa gttccagctc gctgccacct cactaacatt tttacaatt 12540
atattgtaaa attcaactct accagggtgt agagccagggt gtggtggctc acacctgtaa 12600
ttccaacaac tccagaggcc aaggcgagag gatcatttga acccacggaa tttgaggctg 12660
tagtgagtca tgatcacgcc attgcactcc atcctgggca acagagttag accctgaata 12720
tttaaaaaca acaacaacaa caaaactcta tcaggatata ataagtactt agagtgaat 12780
acttgcactc gtaatagaga cttatttttt ttttttttga gacacagtct caccctgttg 12840

```

```

cccaggctgg agtgcaagtgg ttgatctctc gctcacggca acctccatct cccaggttca 12900
agtgagttcc cattcctcag cccagagct gggaccacag gcgcgcgaat ttttgatatt 12960
ttagcagaga cggggtttca ctatgttggc caggctagtc tcaaactcaa gttggcctca 13020
agtgatctgc ccaccctggc gtcccagtggt tgggatttca ggcatgagcc actgtgcctg 13080
gccatgtaat agagactttt aatataggag ggtgtaccag aagcaccagt ttcctgtggc 13140
aaacagaatt attcctgctg tatttgtaat ttggtgccac gaggtagccc agatcccttc 13200
agctctgatg gaagagcatt gcttcagccg taaatggaca cctgcagaaa ccttgaccg 13260
atggatagtc tccctcagct cegtgccatc gctgcagggg ctgttatgga catcactgca 13320
gcccagtggtc tctctctcct ggtctccacc atatgagttg gcttctgttt ctctcctgtt 13380
ttactttgcc tttagctgtg gtctttcaaa ccaccatccc tcttatctt cctctgctgg 13440
ttcctcagat cttcctctga tggcgctgcc tccatgccat gccctctgcc agttctatgt 13500
ggtgaacagt gagctgtcct gccagctgta ccagagatcg ggagacatgg gcctcggtgt 13560
gcctttcaac atcgccagct acgccctgct cacgtacatg attgcgca caacgggct 13620
gaagggtgggc tgtctcgga aggggtgactt gccagcctac cacatgagct cttcagttct 13680
ttaatatggg aaaacaaatt gcagagttaa gtctctgatt agcttttaaa tttgatattg 13740
gtaagtaaga catgaaccag cttttacttt gaaaccttc tttctggaa ggttttctgg 13800
ccctgtggta tatgactaa cagatctata caggttgttt gtgatacagc ttctatggat 13860
cttctcaaaa gctatgctga ggttgggtat ggtggctcat gcctgtaatc ccagcacttt 13920
ggaagactga gacaggagca attgcttgag gtctggagtt caataccagc ctgggcaaca 13980
taacaagatg ctgttgctac aaaaaaatgg aaaagctaca ctaaattatt ttttaaaaa 14040
aagccttgcg gtgtctgcat attctaattgt ttttaaatga tgttttaag aattgaaact 14100
aacatactgt tctgctttct cccggtttat agccagggtga ctttatacac actttgggag 14160
atgcacatat ttacctgaat cacatcgagc cactgaaaat tcaggtaaga attagatgtt 14220
atacttttgg gtttgggtacc ttctcttgat aaaagggtga ctgtggaaca ggtatctgct 14280
caatctgtgt tccaagataa agatgactgc tccaaatgtg gggcttcagt ttagggagaa 14340
gtggtgggca ggtgggcagg acaaggcagg catctgcctc agcaaccatg gcacttaact 14400
tgtcaggtgc tgtgaggtac taagcaccag taccagagag ggaagagcca cattcaagcc 14460
aggggattgt ccaaaaggag gcattttaac tcattttaac ttgaaggaga attgaagtgc 14520
aaatgttttt cttttcttt ttttttgaga tggagtcttt ctctgtcggc caggctggag 14580
tgtgccgtgg tgcgatctca gctcactgca acctccacct cccgggttca agcaattctt 14640
ctgcctcagc ctcccaggta gctgggatta caggcacatg ccaccacacc cagctaattt 14700
tttgatttat tagtagagat ggggtttcgt catgttggcc aggtgatct caaactcctg 14760
acttcaagt taccacctgc ctccagctcc gaaagtctg gaattacagg cataagccac 14820
caccctggcc ataaatattt ttgtttaatt ttacattaag tacaatattt aggtccaaac 14880
ttcaaaagtc tgttgaaatc cctgaagtta tagcagccaa caattgatat gaaatggcaa 14940
taaaaatgta agttcatctg cttcatgagc cttaaggaaa aaaactcaga accagacact 15000
tttttagcccc ttccagggtta gatccagggt ttaaaagtta ttcttttgag ggagtttggc 15060
tgcttttgag tggagggtgac ttcaggctta ttctctctgg ctctctgctc tggctatttt 15120
tagacatagt aatagggtgt gacctgtctt cacatcctaa ttgccactgt ctgttcatcc 15180
caggaatcct ggctttcatc ctttctgtt cactgtccat gcatgtcatc tttccttctt 15240
tctgccaggg accagatggg ttagggattg tgaattcaag taaacgtaga gctactatga 15300
gttacagatt gactgtgttc ctgtctttaa taaatttgcc aagagtgggt ataagaactt 15360
acacctgatg aggcaccagg ctctgatgc tgtgtaatgt caaaaatac cctcactct 15420
cgatctgtgc aagagaacag ctggttgccg tccaatcatg ttacataacc tacgcgaagg 15480
tatcgacagg atcatactcc tgtaaaatag aactttgttg atcacatcct gtgtacttgt 15540
ttcacggaca tgaggagcaa ttacaacagg tcgtacaatt atggcaaaat aatggcctta 15600
ttttgttttt agcttcagcg agaaccaga ctttcccaa agctcaggat tcttcgaaaa 15660
gttgagaaaa ttgatgactt caaagctgaa gactttcaga ttgaagggtta caatccgcat 15720
ccaactatta aaatggaaat ggctgttttag ggtgctttca aaggagctcg aaggatattg 15780
tcagtcttta ggggttgggc tggatgccga ggtaaaagtt ctttttgctc taaaagaaaa 15840
aggaactagg tcaaaaatct gtccgtgacc tatcagttat taatttttaa ggatgttgcc 15900

```

```

actggcaaat gtaactgtgc cagttctttc cataataaaa ggctttgagt taactcactg 15960
aggggtatctg acaatgctga ggttatgaac aaagtggagga gaatgaaatg tatgtgctct 16020
tagcaaaaac atgtatgtgc atttcaatcc cacgtactta taaagaaggt tgggtgaattt 16080
cacaagctat ttttggaata tttttagaat attttaagaa tttcacaagc tattccctca 16140
aatctgaggg agctgagtaa caccatcgat catgatgtag agtgtgggta tgaactttaa 16200
agttatagtt gttttatatg ttgctataat aaagaagtgt tctgcattcg tccacgcttt 16260
gttcattctg tactgccact tatctgctca gttccttcct aaaatagatt aaagaactct 16320
ccttaagtaa acatgtgctg tattctgggt tggatgctac ttaaaagagt atattttaga 16380
aataatagtg aatatatttt gccctatttt tctcatttta actgcatctt atcctcaaaa 16440
tataatgacc atttaggata gagttttttt tttttttttt taaactttta taaccttaaa 16500
gggttatttt aaaataatct atggactacc attttgccct cattagcttc agcatgggtg 16560
gacttctcta ataatatgct tagattaagc aaggaaaaga tgcaaaacca cttcgggggt 16620
aatcagtgaa atatttttcc ctctgttgca taccagatac ccccggtgtt gcacgactat 16680
ttttattctg ctaatttatg acaagtgtta aacagaacaa ggaattattc caacaagtta 16740
tgcaacatgt tgcttatttt caaattacag tttaatgtct aggtgccagc ccttgatata 16800
gctatttttg taagaacatc ctcttgact ttgggttagt taaatctaaa cttatttaag 16860
gattaagtag gataacgtgc attgatttgc taaaagaatc aagtaataat tacttagctg 16920
attcctgagg gtggtatgac ttctagctga actcatcttg atcggtagga ttttttaaat 16980
ccatttttgt aaaactattt ccaagaaatt ttaagccctt tcacttcaga aagaaaaaag 17040
ttgttggggc tgagcactta attttcttga gcaggaagga gtttcttcca aacttcacca 17100
tctggagact ggtgtttctt tacagattcc tccttcattt ctgttgagta gccgggatcc 17160
tatcaaaagc caaaaaaatg agtctgttta acaaccacct ggaacaaaaa cagattttat 17220
gcatttatgc tgctccaaga aatgctttta cgtctaagcc agaggcaatt aattaatttt 17280
tttttttttg acatggagtc actgtccgtt gccaggctg cagtgcagtg cgcgaatctt 17340
ggctcactgc aacctccacc tcccaggttc aagtgtattt cctgcctcag cctcccatgt 17400
agctgggatc acaggcacct gccaccatgc cgggctaatt ttttgtattt tttgtagaga 17460
cagggtttca ccatgttggc caggctgggtc tcaaacacct gacctcaaat gatccacctg 17520
cctcagcctc ccaaagtgtt gggattacag gcgtaagcca ccatgcccag ccctgaatta 17580
atatttttaa aataagtttg gagactgttg gaaataatag ggcagaggaa catattttac 17640
tggtactctg ccagagttag ttaactcatc aaactctttg ataatagttt gacctctgtt 17700
ggtgaaaatg agccatgata tcttgaacat gatcagaata aatgccccag ccacacaatt 17760
gtagtccaaa ctttttaggt cactaacttg ctatagtgtg ccaggttttt ttgcacaagg 17820
agtgc aaatg ttaagatctc cactagttag gaaaggctag tattacagaa gccttgtcag 17880
aggcaattga acctccaagc cctggccctc aggcctgagg attttgatac agacaaactg 17940
aagaaccgtt tgtagtgga tattgcaaac aaacaggagt caaagcttgg tgctccacag 18000
tctagtccac gagacaggcg tggcagtggtc tggcagcatc tcttctcaca ggggccctca 18060
ggcacagctt accttgggag gcatgtagga agcccgtgtg atcatcacgg gatacttgaa 18120
atgctcatgc agtggtgcaa catactcaca caccctagga ggagggaatc agatcggggc 18180
aatgatgcct gaagtcatat tattcacgtg gtgctaactt aaagcagaag gagcgagtac 18240
cactcaattg acagtgttgg ccaaggctta gctgtgttac catgctttc taggcaagtc 18300
cctaaacctc tgtgcctcag gtccctttct tctaaaatat agcaatgtga ggtggggact 18360
ttgatgacat gaacacacga agtccctctg agaggttttg tgggtgccct taaaagggat 18420
caattcagac tctgtaaaata tccagaatta tttgggttcc tctggtcaaa agtcagatga 18480
atagattaaa atcaccacat tttgtgatct atttttcaag aagcgtttgt attttttcat 18540
atggctgcag cagctgccag gggcttgggg tttttttggc aggtagggtt gggagg 18596

```

<210> 12

<211> 3291

<212> DNA

<213> Homo sapiens

<400> 12

```

accgggcaag cgggaaccag gtggccaccc ggtgtcggtt tcattttcct ttggaatttc 60
tgctttacag acagaacaat ggcagcccga gtacttataa ttggcagtgaggaggaggaa 120
catagcgtgg cctggaaact tgcacagtct catcatgtca aacaagtgtt ggttgcccca 180
ggaaacgcag gcaactgcctg ctctgaaaag atttcaaata ccgccatctc aatcagtgac 240
cacactgccc ttgctcaatt ctgcaaagag aagaaaattg aatttgtagt tgttgacca 300
gaagcacctc tggctgctgg gattgttggg aacctgaggt ctgcaggagt gcaatgcttt 360
ggcccaacag cagaagcggc tcagttagag tccagcaaaa ggtttgccaa agagtttatg 420
gacagacatg gaatcccaac cgcacaatgg aaggctttca ccaaacctga agaagcctgc 480
agcttcattt tgagtgcaga ctccctgct ttggttgta aggccagtg tcttgagct 540
ggaaaagggg tgattgttgc aaagagcaaa gaagagcct gcaaagctgt acaagagatc 600
atgcaggaga aagcctttgg ggcagctgga gaaacaattg tcattgaaga acttcttgac 660
ggagaagagg tgcgtgtct gtgtttcact gatggcaaga ctgtggcccc catgccccca 720
gcacaggacc ataagcgatt actggaggga gatggtggcc ctaacacagg gggaatggga 780
gcctattgtc cagccctca ggtttcta atgatctattac taaaaattaa agatactgtt 840
cttcagagga cagtggatgg catgcagcaa gaggtactc catatacagg tattctctat 900
gctggaataa tgctgaccaa gaatggccc aaagtcttag agtttaattg ccgttttggg 960
gatccagagt gccaaagta cctcccact cttaaaagt atctttatga agtgattcag 1020
tccaccttag atggactgct ctgcacatct ctgacctgtt ggctagaaaa ccacaccgc 1080
ctaactgttg tcatggcaag taaaggttat cctggagact acaccaaggg ttagagata 1140
acagggtttc ctgaggctca agctctagga ctggaggtgt tccatgcagg cactgccctc 1200
aaaaatggca aagtagtaac tcatgggggt agagtctctg cagtcacagc catccgggaa 1260
aatctcatat cagcccttga ggaagccaag aaaggactag ctgctataaa gtttgaggga 1320
gcaatttata ggaaagacgt cggctttcgt gccatagctt tcctccagca gcccaggagt 1380
ttgacttaca aggaatctgg agtagatata gcagctggaa atatgctggg caagaaaatt 1440
cagcctttag caaaagccac ttccagatca ggctgtaaag ttgatcttgg aggttttgct 1500
ggcttttttg atttaaaagc agctgggttc aaagatcccc ttctggcctc tggaacagat 1560
ggcgttgga ctaaactaaa gattgcccag ctatgcaata aacatgatac cattggtcaa 1620
gatttggtag caatgtgtgt taatgatatt ctggcacaag gagcagagcc cctctcttc 1680
cttgattact ttctctgtgg aaaacttgac ctcagtgtaa ctgaagctgt tgttgctgga 1740
attgctaaaag cttgtggaag agctggatgt gctctcctg gaggtgaaac agcagaaatg 1800
cctgacatgt atccccctgg agagtatgac ctagctgggt ttgccgttgg tgccatggag 1860
cgagatcaga aactccctca cctggaaaga atcactgagg gtgatgttgt tgttggaata 1920
gcttcatctg gtcttcatag caatggattt agccttgtga ggaaaatcgt tgcaaaatct 1980
tcctccagt actcctctcc agcacctgat ggttgtgggt accagacttt aggggactta 2040
cttctcacgc ctaccagaat ctacagccat tcaactgtac ctgtcctacg ttccaggacat 2100
gtcaaagcct ttgccatat tactggtgga ggattactag agaactccc cagagtcctc 2160
cctgagaaac ttggggtaga tttagatgcc cagacctgga ggatccccag ggttttctca 2220
tggttgcagc aggaaggaca cctctctgag gaagagatgg ccagaacatt taactgtggg 2280
gttggcgctg tccttgtggg atcaaaggag cagacagagc agattctgag ggatatccag 2340
cagcacaagg aagaagcctg ggtgattggc agtgtgtgtg cacgagctga aggttcccca 2400
cgtgtgaaag tcaagaatct gattgaaagc atgcaataaa atgggtcagt gttgaagaat 2460
ggctccctga caaatcattt ctcttttgaa aaaaaaagg ccagagtggc tgtcttaata 2520
tctggaacag gatcgaacct gcaagcactt atagacagta ctcggaacc aaatagctct 2580
gcacaaattg atattgttat ctccaacaa gccgcagtag ctgggttaga taaagcggaa 2640
agagctggta ttcccactag agtaattaat cataaactgt ataaaaatc tgtagaattt 2700
gacagtgcaa ttgacctagt ccttgaagag ttctccatag acatagtctg tcttgagga 2760
ttcatgagaa ttctttctgg cccctttgtc caaaagtgga atggaaaaat gctcaatata 2820
caccatcct tgctcccttc ttttaagggt tcaaatgcc atgagcaagc cctggaaacc 2880
ggagtacag ttactgggtg cactgtacac tttgtagctg aagatgtgga tgctggacag 2940
attattttgc aagaagctgt tcccgtaag aggggtgata ctgtcgcaac tctttctga 3000

```

```

agagtaaaat tagcagaaca taaaatattt cctgcagecc ttcagctggg gccagtgga 3060
actgtacagc ttggagaaaa tggcaagatc tggtgggtta aagaggaatg aagcctttta 3120
attcagaaat ggggccagtt tagaaagaat tatttgctgt ttgcatgggt gttttttatc 3180
atggacttgg cccaaaagaa aaactgctaa aagacaaaaa agacctcacc cttacttcat 3240
ctattttttt aataaataga gactcactaa aaaaaaaaaa aaaaaaaaaa a 3291

```

```

<210> 13
<211> 1776
<212> DNA
<213> Homo sapiens

```

```

<400> 13
atggtgccct ccagcccagc ggtggagaag caggtgcccg tggaaacctg gcctgacccc 60
gagctccggg cctggcgggc cctcgtgtgc tacctttgct tctacggctt catggcgagc 120
atacggccag gggagagctt catcaccccc tacctcctgg ggcccgacaa gaacttcacg 180
cgggacgagg tcacgaacga gatcacgcgc gtgctgtcgt actcctacct ggccgtgctg 240
gtgcccgtgt tcctgtcac cgactacctg cgctacacgc cgggtgctgt gctgcagggg 300
ctcagcttcg tgctgggtgt gctgctgtgt ctgctgggccc actcgggtggc gcacatgcag 360
ctcatggagc tcttctacag cgtcaccatg gccgcgcgca tcgcctattc ctctacatc 420
ttctctctcg tgcggcccgc gcgctaccag cgtgtggccc gctactcgcg cgctgcgggtg 480
ctgctggggc tggttcaccag ctccgtgctg ggccagctgc tggctcactgt gggccgagtc 540
tccttctcca cgctcaacta catctcgtgt gccttctca ccttcagcgt ggtcctcgcc 600
ctcttcctga agcgccccaa gcgcagcctc ttcttcaacc gcgacgaccg ggggcgggtg 660
gaaacctcgg cttcggagct ggagcgcgat aatcctggcc caggcgggaa gctgggacac 720
gccctgcggg tggcctgtgg ggactcagtg ctggcgcgga tgctgcggga gctgggggac 780
agcctgcggc ggccgcagct gcgcctgtgg tccctctggt ggggtcttcaa ctcgcccggc 840
tactacctgg tggctacta cgtgcacatc ctgtggaacg aggtggacc caccaccaac 900
agtgcgcggg tctacaacgg gcgcgcagat gctgcctcca cgctgctggg cgccatcacg 960
tccttcgccc cgggcttcgt gaagatccgc tgggcgcgct ggtccaagct gctcatcgcg 1020
ggcgtcacgg ccacgcaggc ggggctgggt tccttcttgg cgcacacgcg ccacccgagc 1080
agcatctggc tgtgctatgc ggccctcgtg ctgttcgcgc gctcctacca gttcctcgtg 1140
cccctgcaca cctttcagat tgcattctct ctgtctaaag agctctgtgc cctggtcttc 1200
ggggtcaaca cgttctttgc caccatcgct aagaccatca tcaacttcat tgtctcggac 1260
gtgcggggcc tgggcctccc ggtccgcaag cagttccagt tatactccgt gtacttctctg 1320
atcctgtcca tcatctactt cttggggggc atgctggatg gcctgcgcga ctgccagcgg 1380
ggccaccacc gcgcgcagcc cccggcccag ggccctgagga gtgccgcgga ggagaaggca 1440
gcacagcgac tgagcgtgca ggacaagggc ctcggaggcc tgcagccagc ccagagcccc 1500
ccgctttccc cagaagacag cctgggggct gtggggccag cctccctgga gcagagacag 1560
agcgacccat acctggcccc ggccccggcc ccgcaggcag ctgaattcct gagcccagtg 1620
acaaccctt cccctgcac tctgtcgtcc gcccagcct caggccctga ggctgcagat 1680
gagacttgte cccagctggc tgtccatcct cctggtgtca gcaagctggg tttgcagtg 1740
cttccaagcg acggtgttca gaatgtgaac cagtga 1776

```

```

<210> 14
<211> 2500
<212> DNA
<213> Homo sapiens

```

```

<400> 14
tgaatgcgcc ggggtcgccc tctccgcctc gccgcagtcg gggcagccgc tgccctcttt 60
tccatgtatc gtccaggatc ccatgacaga ttctgttgte acgtctcctt acagagtttg 120

```

```

agcgggtgctg aactgtcagc acatctgtcc ggtccagcat gccttctgag acccccaggg 180
cagaagtggg gccacagc tgccccacc gtcaggggcc acactcggcg aaggggagcc 240
tgagagaagg gtccccagag gataaggaag ccaaggagcc cctgtggatc cggcccgatg 300
ctccgagcag gtgcacctgg cagctggggc ggctgcctc cgagtcccca catcaccaca 360
ctgccccggc aaaatctcca aaaatcttgc cagatattct gaagaaaatc ggggacaccc 420
ctatggctcag aatcaacaag attgggaaga agttcggcct gaagtgtgag ctcttggcca 480
agtgtgagtt cttcaacgcg ggcgggagcg tgaaggaccg catcagcctg cggatgattg 540
aggatgctga gcgcgacggg acgctgaagc ccggggacac gattatcgag ccgacatccg 600
ggaacaccgg gatcgggctg gccctggctg cggcagttag gggctatcgc tgcacatcgc 660
tgatgccaga gaagatgagc tccgagaagg tggactgtg gggggcactg ggggtgaga 720
ttgtgaggac gccaccaat gccaggttcg actccccgga gtcacacgtg ggggtggcct 780
ggcggctgaa gaacgaaatc cccaattctc acatcctaga ccagtaccgc aacgccagca 840
acccctggc tctactacgac accaccgctg atgagatcct gcagcagtg gatgggaagc 900
tgacatgct ggtggcttca gtgggcacgg gcggcaccat caggggcatt gccaggaagc 960
tgaaggagaa gtgtcctgga tgcaggatca ttgggtgga tcccgaaggg tccatcctcg 1020
cagagccgga ggagctgaac cagacggagc agacaaccta cgaggtggaa gggatcggct 1080
acgacttcat cccacggctg ctggacagga cgggtgtgga caagtgggtc aagagcaacg 1140
atgaggaggc gttcaccttt gcccgcagtc tgatcgcgca agaggggctg ctgtgcggtg 1200
gcagtgtgg cagcacggcg gcggtggccg tgaaggctgc gcaggagctg caggaggggc 1260
agcgtgcgt ggtcattctg cccgactcag tgcggaacta catgaccaag ttcttgagcg 1320
acaggtgga gctgcagaag ggcttcttga aggaggagga cctcacggag aagaagccct 1380
ggtggtggca cctccgtgtt caggagctgg gcctgtcagc cccgctgacc agtctccga 1440
ccatcacctg tgggcacacc atcgagatcc tccgggagaa gggcttcgac caggcgcccg 1500
tggtggatga ggcgggggta atcctgggaa tggtagcgt tgggaacatg ctctcgtccc 1560
tgcttgccgg gaaggtgcag ccgtcagacc aagtggcaa agtcatctac aagcagttca 1620
aacagatccg cctcacggac acgctgggca ggctctcgca catcctggag atggaccact 1680
tcgccctggt ggtgcacgag cagatccagt accacagcac cgggaagtc agtcagcggc 1740
agatggtgtt cggggtggtc accgccattg acttgttgaa ctctcgtggc gccaggagc 1800
gggaccagaa gtgaagtccg gagcgctggg cgggtcggag cgggcccgc acccttgc 1860
acttctcctt gccttctctg agccctaaac acacgcgtga ttggttaact cctggcctgg 1920
caccgttata cctgcagacg gcacagagca tccgtctccc ctcgtaaca catggcttcc 1980
taaattggcc tgtttacggc ctatgagatg aaatatgtga ttttctctaa tgtaacttcc 2040
tcttaggatg tttaccaag gaaatatga gagagaagtc ggccaggtag gatgaacaca 2100
ggcaatgact gcgcagagtg gattaaaggc aaaagagaga agagtccagg aagggcggg 2160
gagaagcctg ggtggctcag catcctccac gggctgcgcg tctgctcggg gctgagctgg 2220
cgggagcagt ttgcgtgttt ggggttttta attgagatga aattcaaata acctaaaaat 2280
caatcacttg aaagtgaaca atcagcggca tttagtacat ccagaaagtt gtgtaggcac 2340
cacctctgtc acgttctgga acattctgtc atcaccctgt gaagcaatca tttccctccc 2400
cgtcttcttc ctcccctggc aactgctgat cgactttgtg tctctgttgt ctaaaatagg 2460
ttttccctgt tctggacatt tcatataaat ggaatcacac 2500

```

<210> 15

<211> 2068

<212> DNA

<213> Homo sapiens

<400> 15

```

cggcagccct cctacctgcg cagctgggtgc cgctgctgct gcctcccgt cgccctgaac 60
ccagtgcctg cagccatggc tcccggccag ctgccttat ttagtgtctc tgacaaaacc 120
ggccttgggg aatttgcaag aaacctgacc gctcttgggt tgaatctggg cgcttccgga 180
gggactgcaa aagctctcag ggatgctggg ctggcagtca gagatgtctc tgagttgacg 240

```

```

ggatttcctg aaatgttggg gggacgtgtg aaaactttgc atcctgcagt ccatgctgga 300
atcctagctc gtaatatcc agaagataat gctgacatgg ccagacttga tttcaatctt 360
ataagagttg ttgcctgcaa tctctatccc ttgttaaaga cagtggcttc tccaggtgta 420
actgttgagg aggctgtgga gcaaattgac attggtggag taaccttact gagagctgca 480
gccaaaaacc acgctcgagt gacagtgggtg tgtgaaccag aggactatgt ggtggtgtcc 540
acggagatgc agagctccga gagtaaggac acctccttgg agactagacg ccagttagcc 600
ttgaaggcat tctactcatac ggcacaatat gatgaagcaa tttcagatta tttcaggaaa 660
cagtacagca aaggcgtatc tcagatgccc ttgagatatg gaatgaaccc acatcagacc 720
cctgcccagc tgtacacact gcagcccaag cttcccatca cagttctaaa tggagcccct 780
ggatttataa acttgtgcga tgctttgaac gcctggcagc tggatgaagg actcaaggag 840
gcttttaggt tccagccgc tgccctcttc aaacatgtca gccagcagg tgctgtgtt 900
ggaattccac tcagtgaaga tgaggccaaa gctgcatgg tttatgatct ctataaaacc 960
ctcacacca tctcagcggc atatgcaaga gcaagagggg ctgataggat gtcttcattt 1020
ggtgattttg ttgcattgtc cgatgtttgt gatgtaccaa ctgcaaaaat tatttccaga 1080
gaagtatctg atggtataat tgccccagga tatgaagaag aagccttgac aatactttcc 1140
aaaaagaaaa atggaaacta ttgtgtcctt cagatggacc aatcttacia accagatgaa 1200
aatgaagttc gaactctctt tggctctcat ttaagccaga agagaaataa tgggtgtcgtc 1260
gacaagtcac tatttagcaa tgttgttacc aaaaataaag atttgccaga gtctgccctc 1320
cgagacctca tcgtagccac cattgtctgc aagtacactc agtctaactc tgtgtgtctac 1380
gccaaagacg ggcaggttat cggcattgga gcaggacagc agtctcgtat acactgcact 1440
cgctctgcag gagataaggc aaactattgg tggcttagac accatccaca agtgccttcg 1500
atgaagttta aaacaggagt gaagagagca gaaatctcca atgccatcga tcaatatgtg 1560
actggaacca ttggcgagga tgaagatttg ataaagtggg aggcactggt tgaggaagtc 1620
cctgagttac tctactgaggc agagaagaag gaatgggttg agaaactgac tgaagtttct 1680
atcagctctg atgccttctt ccttttccga gataacgtag acagagctaa aaggagtggt 1740
gtggcgtaga ttgctggtcc ctccggttct gctgctgaca aagttgtgat tgaggcctgc 1800
gacgaactgg gaatcatcct cgctcatacg aaccttcggc tcttccacca ctgattttac 1860
cacacactgt tttttggctt gcttatgtgt aggtgaacag tcacgcctga aactttgagg 1920
ataacttttt aaaaaataaa aacagtatct cttaaaacaa tgttttgatc tacataaaca 1980
ttgtaaaaat tttcaatcac gctttttaac tttcttacca caaaaaatg ataagtgggt 2040
gaagtgatgg ttatgttaat tagcgtgc 2068

```

<210> 16

<211> 857

<212> DNA

<213> Homo sapiens

<400> 16

```

gcgtgggctg gagatggcgg cggcagcggg gagcagcgcc aagcggagcc tgcggggaga 60
gctgaagcag cgtctgcggg cgatgagtg caggagcgg ctacgccagt cccgcgtact 120
gagccagaag gtgattgccc acagtgagta tcaaaagtcc aaaagaattt ccattcttct 180
gagcatgcaa gatgaaattg agacagaaga gatcatcaag gacattttcc aacgaggcaa 240
aatctgcttc atccctcggg accggttcca gagcaatcac atggatatgg tgagaataga 300
atcaccagag gaaatttctt tacttcccaa aacatcctgg aatatccctc agcctggtga 360
gggtgatgtt cgggaggagg ccttgtccac agggggactt gatctcatct tcatgccagg 420
ccttgggttt gacaaacatg gcaaccgact ggggaggggc aagggtact atgatgccta 480
tctgaagcgc tgtttgcagc atcaggaagt gaagccctac accctggcgt tggctttcaa 540
agaacagatt tgccctcagg tccagtgaa tgaaaacgac atgaaggtag atgaagtcct 600
ttacgaagac tcgtcaacag cttaaatctg gattactaca gccaaataat cagtgtttta 660
tatgagagta aagcaaagta tgtgtatttt tcccttgtca aaaattagtt gaaattgttc 720
attaatgtga atacagactg cattttaaaa ttgtaattat gaaatacctt atataaaacc 780

```

atcttttaaaa accaatagaa gtgtgaatag tagaatatta attaaaatgg aggctatcag 840
cctgtgattt tcagctt 857

<210> 17

<211> 3762

<212> DNA

<213> Homo sapiens

<400> 17

```

cccgcgagcg tccatccatc tgtccggccg actgtccagc gaaaggggct ccaggccggg 60
cgcacgtcga cccgggggac cgaggccagg agagggggcca agagcgcggc tgacccttgc 120
gggcccggggc aggggacggg ggccgcggcc atgcagtcct gtgccagggc gtgggggctg 180
cgcctggggc gcgggggtcg gggcgggccg cgcttgctg ggggatcggg gccgtgctgg 240
gcgcgcggga gccgggacag cagcagtggc ggccggggaca gcgcccgggc tggggcctcg 300
cgctccttgc agcgcttct gccagacac gacgacttcg ctcgaggca catcgccct 360
ggggacaaag accagagaga gatgctgcag accttggggc tggcgagcat tgatgaattg 420
atcgagaaga cggtccttgc caacatccgt ttgaaaagac ccttgaaaat ggaagaccct 480
gtttgtgaaa atgaaatcct tgcaactctg catgccattt caagcaaaaa ccagatctgg 540
agatcgtata ttggcatggg ctattataac tgctcagtgc cacagacgat tttgcggaac 600
ttactggaga actcaggatg gatcaccag tatactccat accagcctga ggtgtctcag 660
gggaggctgg agagtttact caactaccag accatgggtg gtgacatcac aggctggac 720
atggccaatg catccctgct ggatgagggg actgcagccg cagaggcact gcagctgtgc 780
tacagacaca acaagaggag gaaatttctc gttgatcccc gttgccacc acagacaata 840
gctgttgtcc agactcgagc caaatatact ggagtcctca ctgagctgaa gttaccctgt 900
gaaatggact tcagtggaaa agatgtcagt ggagtgtgt tccagtaccc agacacggag 960
gggaaggtgg aagactttac ggaactcgtg gagagagctc atcagagtgg gagcctggcc 1020
tgctgtgcta ctgacctttt agctttgtgc atcttgaggc cacctggaga atttggggta 1080
gacatcgccc tgggcagctc ccagagattt ggagtgccac tgggctatgg gggaccccat 1140
gcagcatttt ttgctgtccg agaaagcttg gtgagaatga tgcctggaag aatggtgggg 1200
gtaacaagag atgccactgg gaaagaagtg tatcgtcttg ctcttcaaac caggagcaaa 1260
cacattcgga gagacaaggc taccagcaac atctgtacag ctcaggccct cttggcgaat 1320
atggctgcca tgtttcgaat ctaccatggt tcccatgggc tggagcata tgcctaggag 1380
gtacataatg ccactttgat tttgtcagaa ggtctcaagc gagcagggca tcaactccag 1440
catgacctgt tctttgatac cttgaagatt cattgtggct gctcagtga ggaggtcttg 1500
ggcagggcgg ctcagcggca gatcaatttt cggctttttg aggatggcac acttggtatt 1560
tctcttgatg aaacagtcaa tgaaaaagat ctggacgatt tgttgtggat ctttggttgt 1620
gagtcactct cagaactggt tgctgaaagc atgggagagg agtgagagg tattccaggg 1680
tctgtgttca agaggaccag cccgttcctc acccatcaag tgttcaacag ctaccactct 1740
gaaacaaaca ttgtccggtg catgaagaaa ctggaaaata aagacatttc cttgtttcac 1800
agcatgattc cactggggtc ctgcaccatg aaactgaaca gttcgtctga actcgacact 1860
atcacatgga aagaatttgc aaacatccac ccctttgtgc ctctggatca agctcaagga 1920
tatcagcagc ttttccgaga gcttgagaag gatttgtgtg aactcacagg ttatgaccag 1980
gtctgtttcc agccaaacag cggagcccag ggagaatatg ctggactggc cactatccga 2040
gcctacttaa accagaaagg agaggggcac agaacgggtt gcctcattcc gaaatcagca 2100
catgggacca acccagcaag tgcccacatg gcaggcatga agattcagcc tgtggagggt 2160
gataaatatg ggaatatcga tgcagttcac ctcaaggcca tgggtggataa gcacaaggag 2220
aacctagcag ctatcatgat tacataccca tccaccaatg ggggtgtttga agagaacatc 2280
agtgcagtgt gtgacctcat ccatcaacat ggaggacagg tctacctaga cggggcaaat 2340
atgaatgctc aggtgggaat ctgtcgccct ggagacttcg ggtctgatgt ctgcaccta 2400
aatcttcaca agaccttctg cattccccac ggaggagggt gtcctggcat ggggcccac 2460
ggagtgaaga aacatctcgc cccgtttttt cccaatcatc ccgtcatttc actaaagcgg 2520

```

```

aatgaggatg cctgtcctgt gggaaccgtc agtgcggccc catggggctc cagttccatc 2580
ttgcccattt cctgggctta tatcaagatg atgggaggca agggctctta acaagccacg 2640
gaaactgcga tattaaatgc caactacatg gccaaagcat tagaaacaca ctacagaatt 2700
cttttcaggg gtgcaagagg ttatgtgggt catgaattta ttttggacac gagacccttc 2760
aaaaagtctg caaatattga ggctgtggat gtggccaaga gactccagga ttatggattt 2820
cacgccccca ccatgtcctg gcctgtggca gggaccctca tgggtggagcc cactgagtcg 2880
gaggacaagg cagagctgga cagattctgt gatgccatga tcagcattcg gcaggaaatt 2940
gctgacattg aggagggccg catcgacccc agggccaatc cgctgaagat gtctccacac 3000
tccctgacct gcgttacatc ttcccactgg gaccggcctt attccagaga ggtggcagca 3060
ttcccactcc ccttcatgaa accagagaac aaattctggc caacgattgc ccggattgat 3120
gacatatatg gagatcagca cctggtttgt acctgccac ccatggaagt ttatgagtct 3180
ccattttctg aacaaaagag ggcgtcttct tagtctctc tccctaagtt taaaggactg 3240
at ttgatgcc tctccccaga gcatttgata agcaagaaag atttcatctc ccaccccagc 3300
ctcaagtagg agttttatat actgtgtata tctctgtaat ctctgtcaag gtaaattgtaa 3360
atacagtagc tggagggagt cgaagctgat ggttggaaga cggattttgt ttggtattct 3420
gcttccacat gtgccagttg cctggattgg gagccatttt gtgttttgcg tagaaagttt 3480
taggaacttt aacttttaat gtggcaagtt tgcagatgtc atagaggcta tcttggagac 3540
ttaatagaca tttttttgtt ccaaaaagat ccactgtggac tgtgccatct gtgggaaatc 3600
ccagggcaaa tgtttacatt ttgtataccc tgaagaactc ttttctctc aatatgccta 3660
atctgtaatc acattttctga gtgttttctc ctttttctgt gtgaggtttt tttttttttt 3720
aatctgcatt tattagtatt ctaataaaaag cattttgatc gg 3762

```

<210> 18

<211> 1192

<212> DNA

<213> Homo sapiens

<400> 18

```

ggctccctcc ggccgcgaac tgccctctcc cgccccgctt cccggcgagg gtggccgagg 60
cgtagcgccg cgacccccgc acccctgcga acatggcgct gcgagtgggt cggagcggtg 120
gggcccctgt ctgcacctg cgcgcggtcc cgttaccgct cgcgccctgc ccgcccaggc 180
cctggcagct ggggggtggg gccgtccgta cgctgcgcac tggaccgct ctgctctcgg 240
tgcgtaaat cagagagaaa cacgaatggg taacaacaga aaatggcatt ggaacagtgg 300
gaatcagcaa ttttgacag gaagcggttg gagatgttgt ttattgtagt ctccctgaag 360
ttgggacaaa attgaacaaa caagatgagt ttggtgcttt ggaaagtgtg aaagctgcta 420
gtgaactata ttctccttta tcaggagaag taactgaaat taatgaagct ctgacagaaa 480
atccaggact tgtaaacaaa tcttgttatg aagatggttg gctgatcaag atgacactga 540
gtaacccttc agaactagat gaacttatga gtgaagaagc atatgagaaa tacataaaat 600
ctattgagga gtgaaaatgg aactcctaaa taaactagta tgaaataacg aagccagcag 660
agttgtctta aattagtggg ggatagagac ttagaataga aacttttagt attaccgatg 720
gggcaaaaaa aaactactgt taacactgct aatgaaagaa aatgcccttt aactttgtaa 780
tgattataga taaatataat atgcgctctt ttcacaatat cctatgattt ttagactagg 840
ctctagtgtt cagaattcat gaaattatcc atggtaaaaa ctagtataaa aaattacata 900
attcaaaagt aacattgtta ttcttaagcc ttatataata ttgtaacttg catgtatcca 960
tacctggatt tgggatgaaa tacttaatga tctttccatt ggaaataact ggaagtgaag 1020
aggttttgtt gcttgtacag tgtcagatga ggaacaccac tatcttaatt ttgcgataca 1080
ctgcatttgc tgggtgctatt ttatatacag gaagcaacag ctttgcagca aaataataaa 1140
atacttcttc gttaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aa 1192

```

<210> 19

<211> 2102

<212> DNA

<213> Homo sapiens

<400> 19

```

tgcccacgcc ccccttcagat cctttgctcc ggagagagac ctgtccgagc agaggcctgg 60
actacatctc ccggcggtgcc tggcagtggt gtggcctctg tgcgccgtct gcactcgttg 120
caggcgacga tgcagagggc tgtaagtgtg gtggcccgtc tgggctttcg cctgcaggca 180
ttccccccgg ccttggtgtg tccacttagt tgcgcacagg aggtgctccg caggacaccg 240
ctctatgact tccacctggc ccacggcggg aaaatgggtg cgtttgcggt ttggagtctg 300
ccagtgcagt accgggacag tcacactgac tcgcacctgc acacacgcca gcactgctcg 360
ctctttgacg tgtctcatat gctgcagacc aagatacttg gtagtgaccg ggtgaagctg 420
atggagagtc tagtggttgg agacattgca gagctaagac caaaccaggg gacactgtcg 480
ctgtttacca acgaggttgg aggcattcta gatgacttga ttgtaaccaa tacttctgag 540
ggccacctgt atgtggtgtc caacgttggc tgctgggaga aagatttggc cctcatgcag 600
gacaaggtca gggagcttca gaaccagggc agagatgttg gcctggaggt gttggataat 660
gccctgctag ctctgcaagg cccactgca gccaggtac tacaggccgg cgtgtctggc 720
gacctgagga aactgccctt catgaccagt gctgtgatgg aggtgtttgg cgtgtctggc 780
tgccgcgtga cccgctgtgg ctacacagga gaggatggtg tggagatctc ggtgccggtg 840
gccccggcag ttcacctggc aacagctatt ctgaaaaacc cagaggtgaa gctggcaggg 900
ctggcagcca gggacagcct gcgcctggag gcaggcctct gcctgtatgg gaatgacatt 960
gatgaacaca ctacacctgt ggagggcagc ctcatgttga cactggggaa gcgccgccga 1020
gctgctatgg acttccctgg agccaaggct attgttcccc agctgaaggg caggggtgcag 1080
cggaggcgtg tggggttgat gtgtgagggg gccccatgc gggcacacag tcccatcctg 1140
aacaatggag gtaccaagat tggtagtggt actagtggct gcccctcccc ctctctgaag 1200
aagaatgttg cgtggttga tggtccctgc gtagtcagtc gtccaggagc aatgctgctg 1260
gtagaggtgc ggcggaagca gcagatggct gtagtcagca agatgccctt tgtgcccaca 1320
aactactata ccctcaagtg aagctggctc aggggtggggc tgtcccttcc aggagttttg 1380
cccctacaag gggtagtca agaagctgag gcagaactca ctgggggtgg gcagttaagg 1440
tggaggctga ttctaattgt ctggttgagg ggccacacca cctattcccc ccacctaaact 1500
catgccattc cagcttcctt caggaccctg cttctgagtg acggaccagc tcacacaatg 1560
tcttggttca gtccatgate cactgacct actcttgccg gctggagggt aatgagaagc 1620
tttggttctg ccatctctcc cactctgcca ggtgctggct gtggagcaaa ggctcacctt 1680
tgtggagagg ataaaacctg cccaacctac ctacacctgg tttttcacat tgcaagggtg 1740
aataacatgg gcagtgcgga cttaggctac cccctccagt ttgctttccg taaatgcaaa 1800
ttgtccttac tgcaagtcag gaatgattgc tgactcacag tagggctgct atgcctgtgt 1860
gtaaaacttg ggatggctga gggaacatag actcactctt ccacattccc aagttggtct 1920
agtgtgctgc ccagttagca accatggcag actcaccacc tattctgagt tccagggtctg 1980
ctgtagggca ggggtgggctt cctcccagac ttgccttacc ctgggctgat ctttggccct 2040
ggtatgcatt aatggactcc actgaatcct gaaaaaaaaa ttaaaacttc ttcttacttg 2100
cc 2102

```

<210> 20

<211> 3228

<212> DNA

<213> Homo sapiens

<400> 20

```

aaaaaactca ggcaaagtca cagcctcaaa attgttcact gaaagaacgc tgagtggaga 60
agtgtgagaa gatgaatgga ccggtggatg gcttgtgtga ccactctcta agtgaaggag 120
tcttcatgtt cacatcggag tctgtgggag agggacaccc ggataagatc tgtgaccaga 180
tcagtgatgc agtgcctggat gccatctca agcaagaccc caatgccaaag gtggcctgtg 240

```

```

agacagtgtg caagaccggc atggtgctgc tgtgtggtga gatcacctca atggccatgg 300
tggactacca gcgggtggtg agggacacca tcaagcacat cggctacgat gactcagcca 360
agggctttga cttcaagact tgcaacgtgc tgggtggcttt ggagcagcaa tccccagata 420
ttgcccagtg cgtccatctg gacagaaatg aggaggatgt gggggcagga gatcagggtt 480
tgatgttcgg ctatgtctacc gacgagacag aggagtgcac gccctcacc atcatccttg 540
ctcacaagct caacgcccgg atggcagacc tcaggcgtc cgccctcctc ccctggctgc 600
ggcctgactc taagactcag gtgacagttc agtacatgca ggacaatggc gcagtcaccc 660
ctgtgcgcat ccacaccatc gtcatctctg tgcagcacia cgaagacatc acgtgagg 720
agatgcgcag ggcctgaag gagcaagtca tcagggccgt ggtgccggcc aagtacctgg 780
acgaagacac cgtctaccac ctgcagccca gtgggcggtt tgtcatcgga ggtccccagg 840
gggatgcggg tgtcactggc cgtaagatta ttgtggacac ctatggcggc tggggggctc 900
atggtggtgg ggccttctct ggggaaggact acaccaaggt agaccgctca gctgcatatg 960
ctgcccgtcg ggtggccaag tctctggtga aagcagggt ctgccggaga gtgcttgtcc 1020
aggtttccta tgccattggt gtggccgagc cgctgtccat ttccatcttc acctacggaa 1080
cctctcagaa gacagagcga gagctgctgg atgtggtgca taagaacttc gacctccggc 1140
cgggcgtcat tgtcagggat ttggacttga agaagcccat ctaccagaag acagcatgct 1200
acggccattt cggaagaagc gagttcccat gggaggttcc caggaagctt gtattttaga 1260
gccaggggga gctgggcctg gtctcaccct ggaggcacct ggtggccatg ctctcttcc 1320
ccagacgcct ggctgctgat cgccttcccc accaccaac cctcagggca aagccaggtc 1380
cctctcattt agcctgtcct gtcatcatca tggccagctg gaggcagggg cttcctggtg 1440
ctggagggtg gatcttgatg taaggatggg catggtgttc tctgctgct ccctcagact 1500
ggggcaatgt taatttagtg gaaaaggcac ccccgtaag agtgaattcc ctactcgtc 1560
tcccccaaca gctggaccct gaccagctcc cctcctctcc ccttgctgt gccagggtgag 1620
gtcagcacat ctcaacagge ctcagggtc ctgtggtggc tgggctcctg gacccccctt 1680
tcacaggcag ccagtgcctt gagccagggt cctcagaaa cccacccag cccaggcatg 1740
tggcagggtt tagagcagga ctgatgtctc ctaagcacct gtaatgtgcg agggaccag 1800
ctaataactg atctcgtttt ttcttactg caacatgatg aggtagtacc ttttatatcc 1860
catttataga tgggggaaag caaagcacag agagtctgga taactccac aggggtccac 1920
agccacgtgt ttagacctag atgtataact aggagcttg actcaggagc ctgtgacata 1980
cccccttccc caccgttgte tcatgccagt aacaggctca aacaatgaca aagcagattc 2040
agaaatgagg ccatggactc tgtcctgaag gcctgaggtt actggaaatt aggggattaa 2100
cccactagct cttgttgagc cgtgggcaat tgtctgaaaa gtgaagacag aaccacaggg 2160
ctattttgtt tgcttcatgt gtcccagaag atgactgagg gtgagttggc ttacctggcc 2220
catcagggtt ggctggagtt agggactgac cagcagcttt agaattcccag cccctgacc 2280
actcagagac atgcagagat tgggtttttg gacttctggg gtaagtggtc taagtccagt 2340
ccagtccat gtgggcttcc tggagcagaa gcagcaactt gtcctagcac agatggccag 2400
ccccctagac agaggccctc aagtctttct ctttccctgg tcccttgat cccctgcagg 2460
ctgagtgcac ttggaggag tgagtggccc ttccggatcc agggaggctg gtcctatggc 2520
ctcatgttaa ataggcgggg cttgccttct ggtgttgga aagcttctga gacgtcatga 2580
ggagattctg cctttgccag gtgactgtct ggggagcggg tctgctccca aggggcctga 2640
gcagtccctg gcctgctaag gtcttggaa cttgcctgct tccatccat ggccagcagc 2700
acctgcccta cctgccccac ttgtccttag cctggacctc tgacagcagc atctctacct 2760
tctccccagc tcccaggacc acaggctcag gcaggccctc catgggcccc aggggaacac 2820
tggggacttg gcctctctct aggggtacatg gtgctgggag aggcagccca ggaagtctca 2880
tctggggagc aggcagccag catctgggcc ttggcctgga gcacaaagac cctggcttcc 2940
atcttctctc aggtgaaagg aaattaaggc aacaaaagaa gcccggtcc tggctacct 3000
ggaagcctca gattccttcc catggaggga gggagtgggt tgcagggtgg caagtccctc 3060
taacttggct cactctgac atgaaaattc agaattttat actttcccta cctctagag 3120
aaataagatc tttttgtca gtttgtttgt atgaaactaa agctttattt gttaatagtt 3180
cctgctaaaa caatgaataa aaactcaagg agcaactaaa aaaaaaaa 3228

```

<210> 21
 <211> 344
 <212> PRT
 <213> Homo sapiens

<400> 21
 Met Ser Ala Leu Ala Ala Arg Leu Leu Gln Pro Ala His Ser Cys Ser
 1 5 10 15
 Leu Arg Leu Arg Pro Phe His Leu Ala Ala Val Arg Asn Glu Ala Val
 20 25 30
 Val Ile Ser Gly Arg Lys Leu Ala Gln Gln Ile Lys Gln Glu Val Arg
 35 40 45
 Gln Glu Val Glu Glu Trp Val Ala Ser Gly Asn Lys Arg Pro His Leu
 50 55 60
 Ser Val Ile Leu Val Gly Glu Asn Pro Ala Ser His Ser Tyr Val Leu
 65 70 75 80
 Asn Lys Thr Arg Ala Ala Ala Val Val Gly Ile Asn Ser Glu Thr Ile
 85 90 95
 Met Lys Pro Ala Ser Ile Ser Glu Glu Glu Leu Leu Asn Leu Ile Asn
 100 105 110
 Lys Leu Asn Asn Asp Asp Asn Val Asp Gly Leu Leu Val Gln Leu Pro
 115 120 125
 Leu Pro Glu His Ile Asp Glu Arg Arg Ile Cys Asn Ala Val Ser Pro
 130 135 140
 Asp Lys Asp Val Asp Gly Phe His Val Ile Asn Val Gly Arg Met Cys
 145 150 155 160
 Leu Asp Gln Tyr Ser Met Leu Pro Ala Thr Pro Trp Gly Val Trp Glu
 165 170 175
 Ile Ile Lys Arg Thr Gly Ile Pro Thr Leu Gly Lys Asn Val Val Val
 180 185 190
 Ala Gly Arg Ser Lys Asn Val Gly Met Pro Ile Ala Met Leu Leu His
 195 200 205
 Thr Asp Gly Ala His Glu Arg Pro Gly Gly Asp Ala Thr Val Thr Ile
 210 215 220
 Ser His Arg Tyr Thr Pro Lys Glu Gln Leu Lys Lys His Thr Ile Leu
 225 230 235 240

Ala Asp Ile Val Ile Ser Ala Ala Gly Ile Pro Asn Leu Ile Thr Ala
245 250 255

Asp Met Ile Lys Glu Gly Ala Ala Val Ile Asp Val Gly Ile Asn Arg
260 265 270

Val His Asp Pro Val Thr Ala Lys Pro Lys Leu Val Gly Asp Val Asp
275 280 285

Phe Glu Gly Val Arg Gln Lys Ala Gly Tyr Ile Thr Pro Val Pro Gly
290 295 300

Gly Val Gly Pro Met Thr Val Ala Met Leu Met Lys Asn Thr Ile Ile
305 310 315 320

Ala Ala Lys Lys Val Leu Arg Leu Glu Glu Arg Glu Val Leu Lys Ser
325 330 335

Lys Glu Leu Gly Val Ala Thr Asn
340

<210> 22

<211> 1283

<212> DNA

<213> Homo sapiens

<400> 22

```

tttcgcagcc gctgccgcct cgccgctgct ccttcgtaag gccacttccg cacaccgaca 60
ccaacatgaa cggacagctc aacggcttcc acgaggcggt catcgaggag ggcacattcc 120
ttttcacctc agagtcggtc ggggaaggcc acccagataa gatttgtgac caaatcagtg 180
atgctgtcct tgatgccac cttcagcagg atcctgatgc caaagtagct tgtgaaactg 240
ttgctaaaac tggaatgatc cttcttgctg gggaaattac atccagagct gctgttgact 300
accagaaagt gggttcgtgaa gctgttaaac acattggata tgatgattct tccaaagggt 360
ttgactacaa gacttgtaac gtgctggtag ccttgaggca acagtcacca gatattgctc 420
aagggtgttca tcttgacaga aatgaagaag acattggtgc tggagaccag ggcttaatgt 480
ttggctatgc cactgatgaa actgaggagt gtatgccttt aaccattgtc ttggcacaca 540
agctaaatgc caaactggca gaactacgcc gtaatggcac ttgacctggg ttacgcctcg 600
attctaaaac tcaagttact gtgcagtata tgcaggatcg aggtgctgtg cttcccatca 660
gagtcacacac aattgttata tctgttcagc atgatgaaga gggttgtctt gatgaaatga 720
gggatgccct aaaggagaaa gtcatacaaag cagttgtgcc tgcgaaatac cttgatgagg 780
atacaatcta ccacctacag ccaagtggca gatttgttat tgggtgggct cagggtgatg 840
ctgggtttgac tggacggaaa atcattgtgg acacttatgg cgggtggggg gctcatggag 900
gaggtgcctt ttcaggaaaag gattatacca aggtcgaccg ttcagctgct tatgctgctc 960
gttggttggc aaaatccctt gttaaaggag gtctgtgccg gaggggttctt gttcagggtc 1020
cttatgctat tggagtttct catccattat ctatctccat tttccattat ggtacctctc 1080
agaagagtga gagagagcta ttagagattg tgaagaagaa ttctgatctc cgccctgggg 1140
tcattgtcag ggatctggat ctgaagaagc caatttatca gaggactgca gctatggcc 1200
actttggtag ggacagcttc ccatgggaag tgcccaaaaa gcttaaatat tgaaagtgtt 1260
agcctttttt ccccagactt gtt 1283

```

<210> 23
 <211> 3259
 <212> DNA
 <213> Homo sapiens

<400> 23
 caagggttggt ggaagtcgcg ttgtgcaggt tcgtgcccgg ctggcgccgc gtggtttcac 60
 tgttacatgc cttgaagtga tgaggagggt tctgttacta tatgctacac agcagggaca 120
 ggcaaaggcc atcgagaag aaatgtgtga gcaagctgtg gtacatggat tttctgcaga 180
 tcttcactgt attagtgaat ccgataagta tgacctaaaa accgaaacag ctctctctgt 240
 tgttgtggtt tctaccacgg gcaccggaga cccacccgac acagcccgcga agtttggttaa 300
 ggaaatacag aaccaaacac tgccgggtga tttctttgct cacctgcggt atgggttact 360
 ggggtctcggg gattcagaat acacctactt ttgcaatggg gggaagataa ttgataaacg 420
 acttcaagag cttggagccc ggcattttcta tgacactgga catgcagatg actgtgtagg 480
 tttagaactt gtggttgagc cgtggattgc tggactctgg ccagccctca gaaagcattt 540
 taggtcaagc agaggacaag aggagataag tggcgcactc ccggtggcat cacctgcac 600
 cttgaggaca gaccttggtga agtcagagct gctacacatt gaatctcaag tcgagcttct 660
 gagattcgat gattcaggaa gaaaggattc tgagggtttg aagcaaaatg cagtgaacag 720
 caaccaatcc aatgttgtaa ttgaagactt tgagtcctca cttaccctgt cggtaccccc 780
 actctcacia gcctctctga atattctctg tttaccccc gaatatttac aggtacatct 840
 gcaggagtct cttggccagg aggaaagcca agtatctgtg acttcagcag atccagtttt 900
 tcaagtgcc aatttcaaagg cagttcaact tactacgaat gatgccataa aaaccactct 960
 gctggtagaa ttggacattt caaatacaga cttttcctat cagcctggag atgccttcag 1020
 cgtgatctgc cctaacagtg attctgaggt acaaagccta ctcaaagac tgcagcttga 1080
 agataaaaaga gagcactgcg tccttttgaa aataaaggca gacacaaaga agaaaggagc 1140
 taccttacct cagcatatag ctgcgggatg tctctccag ttcattttta cctggtgtct 1200
 tgaaatccga gcaattccta aaaaggcatt tttgcgagcc cttgtggact ataccagtga 1260
 cagtgtgaa aagcgcaggc tacaggagct gtgcagtaaa caaggggcag ccgattatag 1320
 ccgctttgta cgagatgcct gtgcctgctt gtggatctc ctctctgctt tcccttcttg 1380
 ccagccacca ctcatctcc tgctcgaaca tcttctaaa cttcaacca gaccatattc 1440
 gtgtgcaagc tcaagtttat ttcacccagg aaagctccat tttgtcttca acattgtgga 1500
 atttctgtct actgccacaa cagaggttct gcggaaggga gtatgtacag gctggctggc 1560
 cttgttggtt gcttcagttc ttcagccaaa catacatgca tcccatgaag acagcgggaa 1620
 agccctggct cctaagatat ccattctctc tcgaacaaca aattctttcc acttaccaga 1680
 tgacccctca atcccatca taatggtggg tccaggaacc ggcatagccc cgtttattgg 1740
 gttcctacaa catagagaga aactccaaga acaacacca gatggaaatt ttggagcaat 1800
 gtggttggtt tttggctgca ggcataagga tagggattat ctattcagaa aagagctcag 1860
 acatttcctt aagcatggga tcttaactca tctaaagggt tcttctcaa gagatgctcc 1920
 tggtggggag gaggaagccc cagcaaagta tgtacaagac aacatccagc ttcattggca 1980
 gcagggtggc agaatcctcc tccaggagaa cggccatatt tatgtgtgtg gagatgcaa 2040
 gaatatggcc aaggatgtac atgatgccct tgtgcaata ataagcaaag aggttggagt 2100
 tgaaaaacta gaagcaatga aaaccctggc cactttaaaa gaagaaaaac gctaccttca 2160
 ggataatttg tcataaaacc agaaattaaa gaaagaggat taagcttttt tgactgaaa 2220
 tactaaaagt cagctttact agtgccaaac ctttaaattt tcaaaagaaa attttctttc 2280
 aacatttctt gaaggacatg gagtgaggat tggatcattt aacaatataa caaaacttcc 2340
 tgatttgatt ttacgtatct tctatctacg cccttctgtt gcctgtgact ctcccaaat 2400
 tgccctgttg ccttgagctc ttctgagcta aaggcagcct tcagtcccta tcagcgctc 2460
 ctttacttcc cagagaactt cacagagact ctgtccttcc atgcaaaggc ttcctgaaat 2520
 aggggagact gactgagtag ctattcttgg tgacttacag tgccaacatt taaaaaagta 2580
 tgaaaatgat ttatttttat atgatgtata ccataaaga atgctcatat taatgtactt 2640
 aaattacaca tgtagagcat atctgttata tgtttatgta actatcaaat ggttatttgt 2700

```

tactaaagct atatttctga taaaaaatat tttaggataa ttgcctacag agggatttat 2760
ttttatgatg ctgggaaata tgaaatgtat tttaaaattt cactctgggc atatggattt 2820
atctatcacc attacttttt tttaagtcac aatttcagaa ttttgggaca tttgcattca 2880
atttacaggt accagtacgt acatatttta atagaaagat acaacctttt tattttcact 2940
cctttttatt ctgctgcttg gcacattttt gagttttccc acattatttg tctccatgat 3000
accactcaag cagtgtgctg gacctaataa actgacttta gttagtatcc ttggattttt 3060
agattcccca gtgtctaatt ccctgttata atttgcacaa acaaaacaaa atgttatgat 3120
aatctttctc cactgttcta atatatattg tatttttatt tgatagcttg ggatttaaaa 3180
catctctgtt gaaggctttt gatccttttg agaaataaag atctgaaaga aatggcataa 3240
tcttaaaaaa aaaaaaaaaa 3259

```

<210> 24

<211> 1805

<212> DNA

<213> Homo sapiens

<400> 24

```

aagagactga actgtatctg cctctatttc caaaagactc acgttcaact ttcgctcaca 60
caaaagccggg aaaattttat tagtctttt tttaaaaaaa gttaatataa aatttataga 120
aaaaaaaaaa ggaacctgaa ctttagtaac acagctggaa caatcgagc ggcggcggca 180
gcggcgggag aagaggttta atttagttga ttttctgttg ttgttggttg ttcgctagtc 240
tcacggtgat ggaagctgca cattttttcg aaggaccga gaagctgctg gaggtttggt 300
tctcccgcca gcagcccgac gcaaaccaag gatctgggga tcttcgcaat atcccaagat 360
ctgagtggga catacttttg aaggatgtgc aatgttcaat cataagtgtg acaaaaactg 420
acaagcagga agcttatgta ctcagtgaga gtagcatgtt tgtctccaag agacgtttca 480
ttttgaagac atgtggtacc accctcttgc tgaaagcact gggtcccttg ttgaagcttg 540
ctagggatta cagtgggttt gactcaattc aaagcttctt ttattctcgt aagaatttca 600
tgaagccttc tcaccaaggg taccacacc ggaatttcca ggaagaaata gagtttctta 660
atgcaatttt cccaaatgga gcaggatatt gtatgggacg tatgaattct gactgttggt 720
acttatatac tctggatttc ccagagagtc gggtaatcag tcagccagat caaaccttgg 780
aaattctgat gagtgagctt gaccagcag ttatggacca gttctacatg aaagatgggt 840
ttactgcaaa ggatgtcact cgtgagagtg gaattcgtga cctgatacca gggtctgtca 900
ttgatgccac aatgttcaat ccttgtgggt attcgatgaa tggaatgaaa tcggatggaa 960
cttattggac tattcacatc actccagaac cagaattttc ttatgttagc tttgaaacaa 1020
acttaagtca gacctcctat gatgacctga tcaggaaagt tgtagaagtc ttcaagccag 1080
gaaaatttgt gaccaccttg tttgttaatc agagttctaa atgtcgaca gtgcttgctt 1140
cgccccagaa gattgaaggt ttttagcgctc ttgattgcca gactgctatg ttcaatgatt 1200
acaattttgt ttttaccagt tttgctaaga agcagcaaca acagcagagt tgattaagaa 1260
aaatgaagaa aaaacgcaaa aagagaacac atgtagaagg tgggtggatgc tttctagatg 1320
tcgatgctgg gggcagtgct ttccataacc accactgtgt agttgcagaa agccctagat 1380
gtaatgatag tgtaatcatt ttgaattgta tgcattatta tatcaaggag ttagatatct 1440
tgcataaatg ctctctcttg tgtttaggta ttctctgcca ctcttgctgt gaaattgaag 1500
tggatgtaga aaaaaccttt tactatatga aactttacaa cacttgtgaa agcaactcaa 1560
tttggtttat gcacagtgt atatttctcc aagtatcctc caaaattccc cacagacaag 1620
gctttcgtcc tcattaggtg ttggcctcag cctaaccctc taggactgtt ctattaaatt 1680
gctgccagaa ttttacatcc agttacctcc actttctaga acatattctt tactaatgtt 1740
attgaaacca atttctactt catactgatg tttttggaaa cagcaattaa agtttttctt 1800
ccatg 1805

```

<210> 25

<211> 254

<212> PRT

<213> Homo sapiens

<400> 25

Gln Asp Ile Leu Val Phe Arg Ser Lys Thr Tyr Gly Asn Val Leu Val
 1 5 10 15

Leu Asp Gly Val Ile Gln Cys Thr Glu Arg Asp Glu Phe Ser Tyr Gln
 20 25 30

Glu Met Ile Ala Asn Leu Pro Leu Cys Ser His Pro Asn Pro Arg Lys
 35 40 45

Val Leu Ile Ile Gly Gly Gly Asp Gly Gly Val Leu Arg Glu Val Val
 50 55 60

Lys His Pro Ser Val Glu Ser Val Val Gln Cys Glu Ile Asp Glu Asp
 65 70 75 80

Val Ile Gln Val Ser Lys Lys Phe Leu Pro Gly Met Ala Ile Gly Tyr
 85 90 95

Ser Ser Ser Lys Leu Thr Leu His Val Gly Asp Gly Phe Glu Phe Met
 100 105 110

Lys Gln Asn Gln Asp Ala Phe Asp Val Ile Ile Thr Asp Ser Ser Asp
 115 120 125

Pro Met Gly Pro Ala Glu Ser Leu Phe Lys Glu Ser Tyr Tyr Gln Leu
 130 135 140

Met Lys Thr Ala Leu Lys Glu Asp Gly Val Leu Cys Cys Gln Gly Glu
 145 150 155 160

Cys Gln Trp Leu His Leu Asp Leu Ile Lys Glu Met Arg Gln Phe Cys
 165 170 175

Gln Ser Leu Phe Pro Val Val Ala Tyr Ala Tyr Cys Thr Ile Pro Thr
 180 185 190

Tyr Pro Ser Gly Gln Ile Gly Phe Met Leu Cys Ser Lys Asn Pro Ser
 195 200 205

Thr Asn Phe Gln Glu Pro Val Gln Pro Leu Thr Gln Gln Gln Val Ala
 210 215 220

Gln Met Gln Leu Lys Tyr Tyr Asn Ser Asp Val His Arg Ala Ala Phe
 225 230 235 240

Val Leu Pro Glu Phe Ala Arg Lys Ala Leu Asn Asp Val Ser
 245 250

<210> 26
 <211> 2211
 <212> DNA
 <213> Homo sapiens

<400> 26
 ctgaggccca gcccccttcg cccggtttcca tcacgagtgc cgccagcatg tctgacaaaac 60
 tgccctacaa agtcgccgac atcggcctgg ctgcctgggg acgcaaggcc ctggacattg 120
 ctgagaacga gatgccgggc ctgatgcgta tgcgggagcg gtactcggcc tccaagccac 180
 tgaagggcgc ccgcatcgct ggctgcctgc acatgaccgt ggagacggcc gtccctcattg 240
 agaccctcgt caccctgggt gctgaggtgc agtgggtccag ctgcaacatc ttctccaccc 300
 agaaccatgc ggcggctgcc attgccaagg ctggcattcc ggtgtatgcc tgggaagggcg 360
 aaacggacga ggagtagcct tggtgcattg agcagaccct gtacttcaag gacggggcccc 420
 tcaacatgat tctggacgac gggggcgacc tcaccaacct catccacacc aagtaccgcg 480
 agcttctgcc aggcattccga ggcatctctg aggagaccac gactgggggc cacaacctct 540
 acaagatgat ggccaatggg atcctcaagg tgccctgcat caatgtcaat gactccgtca 600
 ccaagagcaa gtttgacaac ctctatggct gccgggagtc cctcatagat ggcatacaagc 660
 gggccacaga tgtgatgatt gccggcaagg tagcgggtgt agcaggctat ggtgatgtgg 720
 gcaagggctg tgcccaggcc ctgcgggggt tgcggagccc cgatcatcat accgagattg 780
 accccatcaa cgcactgcag gctgccatgg agggctatga ggtgaccacc atggatgagg 840
 cctgtcagga gggcaacatc tttgtcacca ccacaggctg tattgacatc atccttgccc 900
 ggtaggtgcc agatgggggg tcccggggag tgaggaggga gggcagagtt gggacagctt 960
 tctgtccctg acaatctccc acggtcttgg gctgcctgac aggcactttg agcagatgaa 1020
 ggatgatgcc attgtgtgta acattggaca ctttgacgtg gagatcgatg tcaagtggct 1080
 caacgagaac gccgtggaga aggtgaacat caagccgcag gtggaccggt atcgggtgaa 1140
 gaatggcgcc cgcattcatc tgctggccga gggctcggctg gtcaacctgg gttgtgccat 1200
 gggccacccc agcttcgtga tgagtaactc cttcaccaac cagggtgatg cgcatatcga 1260
 gctgtggacc catccagaca agtaccctgt tgggggttcat ttcctgccc aagaagctgga 1320
 tgaggcagtg gctgaagccc acctgggcaa gctgaatgtg aagttgacca agctaactga 1380
 gaagcaagcc cagtacctgg gcatgtcctg tgatggcccc ttcaagccgg atcactaccg 1440
 ctactgagag ccaggtctgc gtttcacct ccagctgctg tccttgccc gggccacct 1500
 ctctcccta agagctaag gcaccaact tgtgattggt ttgtcagtg ccccatcga 1560
 ctctctgggg ctgatcactt agtttttggc ctctgctgca gccgtcatac tgttccaaat 1620
 gtggcagcgg gaacagagta ccctcttcaa gccccggtca tgatggagggt cccagccaca 1680
 gggaaccatg agctcagtgg tcttggaaaca gctcactaag tcagtccttc cttagcctgg 1740
 aagtcagtag tggagtcaca aagcccatgt gttttgccat ctaggccttc acctggctctg 1800
 tggacttata cctgtgtgct tggtttacag gtccagtgggt tcttcagccc atgacagatg 1860
 agaaggggct atattgaagg gcaaagagga actgttgttt gaattttcct gagagcctgg 1920
 cttagtgtct ggccctctct taaacctcat tacaatgagg ttagtacttt tagtcctgt 1980
 tttacagggg ttagaataga ctgttaagg gcaactgaga aagaacagag aagtacagc 2040
 taggggttga gaggggccag aaaaacatga atgcaggcag atttcgtgaa atctgccacc 2100
 actttataac cagatggttc ctttcacaac cctgggtcaa aaagagaata atttggccta 2160
 taatgttaaa agaaagcagg aaggtgggta aataaaaatc ttggtgcctg g 2211

<210> 27
 <211> 2436
 <212> DNA
 <213> Homo sapiens

<400> 27

```

cgaccacctg tctggacacc acaaagatgc caccctgttg gggcaaaaag gccaaagaagg 60
gcatcctaga acgttttaaat gctggagaga ttgtgattgg agatggaggg tttgtctttg 120
cactggagaa gaggggctac gtaaaggcag gaccctggac tcctgaagct gctgtggagc 180
acccagaagc agttcgccag cttcatcgag agttcctcag agctggctca aacgtcatgc 240
agaccttcac cttctatgcg agtgaagaca agctggagaa caggggcaac tatgtcttag 300
agaagatata tgggcaggaa gtcaatgaag ctgcttgca catcgcccga caagtggctg 360
atgaaggaga tgctttggta gcaggaggag tgagtcagac accttcatac cttagctgca 420
agagtgaac tgaagtcaaa aaagtatttc tgcaacagtt agaggtcttt atgaagaaga 480
acgtggactt cttgattgca gagtattttg aacacgttga agaagctgtg tgggcagttg 540
aaaccttgat agcatccggt aaacctgtgg cagcaacat gtgcattggc ccagaaggag 600
atttgcattg cgtgcccccc ggcgagtggt cagtgcgcct ggtgaaagca ggagcatcca 660
tcattgggtg gaactgccac tttgacccca ccattagttt aaaaacagtg aagctcatga 720
aggagggtt ggaggctgcc caactgaaag ctacactgat gagccagccc ttggcttacc 780
acactcctga ctgcaacaag cagggattca tcgatctccc agaattccca tttggactgg 840
aaccagagt tgccaccaga tgggatattc aaaaatacgc cagagaggcc tacaacctgg 900
gggtcaggta cattggcggg tgctgtggat ttgagcccta ccacatcagg gcaattgcag 960
aggagctggc ccagaaaagg ggctttttgc caccagcttc agaaaaacat ggcagctggg 1020
gaagtggttt ggacatgcac accaaaacct gggtttagagc aagggccagg aaggaatact 1080
gggagaattc tcggatagcc tcaggccggc catacaacct ttcaatgtca aagccagatg 1140
gctggggagt gaccaaagga acagccgagc tgatgcagca gaaagaagcc acaactgagc 1200
agcagctgaa agagctcttt gaaaaacaaa aattcaaatac acagtagcct cgatagaagc 1260
tatttttgat gaatttctag gtgtttgggt cacagttcct acaaatacgg aaaagggggt 1320
taaaaagcag tgctttcatg aatgccatcc tacacatatt attgctatta cctgaacaaa 1380
atagaattac aaatagcact tgataatttt aaagtatgtt ttagaaattt tcttaggagc 1440
aaaataagta caaagtaaat cttgaacagg ttactaagc acccaccctg tgaaaagtat 1500
tatggaaatc actgcagcac aggaaaagta attcagatgt taatgccact tgaagaagtt 1560
ggtagcagc caaagaggat gagacatgaa ctgtcataaa ggactcagca accagccagg 1620
gacagataaa gcgctatgga aaggggcttc caagtctctt tgaacatgac ccttagtaac 1680
aaacacaatt tatataatga ccagcaaaa cacatcacat cttactgtcg aaattaaatg 1740
tgtgatccat cctagtattt tctgttccat tccttttcat tctatttcat ttataaaaca 1800
tgctagttag gacttttcaa atggattttt atgaccact actgggtttg gatccacagt 1860
ttgaaaaata ttgctacaag aactttaagg agaccatcct gtttaagttt attcttataa 1920
gtaggctcag catatgagac ctgatcaata aatatccaat acccagagtc ctgctctcag 1980
agttcttctg tttcgtgacc cacttttcta ccagtaaaag acatagacca atggggagga 2040
ggggaggaga gatggatatt tcagccctct ccactcagc caacactgga tccacctagt 2100
gcctctgggc cataaggctg agcagagtga gcttgtagta gttggtagct tttaaaaaat 2160
ataataaaaa aaaagtagag attctccaaa cctcagcctg gtttcctaga ttgagaacta 2220
tgatattttt ctctgataat ttaatatcta ctctcctaca aaagctcaag cctgaagata 2280
caagactatt agaagaaaca tgactaccct cagtgtatta gaaaagaggt catgcagctt 2340
tctaaacatt attgaattgt ttgagctgtt ttgaaattgt aattcttttc agctattaaa 2400
aagaagagca atgagaaaaa aaaaaaaaaa aaaaaa 2436

```

<210> 28

<211> 1326

<212> DNA

<213> Homo sapiens

<400> 28

```

ttcttttctt ctcttcttct ttcgcggttc agcatgcagg aaaaagacgc ctcttcacaa 60
ggtttctctg cacacttcca acatttcgcc acgcaggcga tccatgtggg ccaggatccg 120

```

```

gagcaatgga cctccagggc tgtagtggcc cccatctcac tgtccaccac gttcaagcaa 180
ggggcgccctg gccagcactc ggggttttgaa tatagccgtt ctggaaatcc cactaggaat 240
tgccttgaaa aagcagtggc agcactggat ggggctaagt actgtttggc ctttgcttca 300
gggttagcag ccactgtaac tattacccat cttttaaaag caggagacca aattatttgt 360
atggatgatg tgtatggagg tacaacagg tacttcaggc aagtggcatc tgaatttggg 420
ttaaagattt cttttgttga ttgttccaaa atcaaattac tagaggcagc aattacacca 480
gaaaccaagc ttgtttggat cgaaaccccc acaaacccca cccagaaggt gattgacatt 540
gaaggctgtg cacatattgt ccataagcat ggagacatta ttttggtcgt ggataacact 600
tttatgtcac catatttcca gcgccctttg gctctgggag ctgatatttc tatgtattct 660
gcaacaaaat acatgaatgg ccacagtgtg gttgtaatgg gcctgggtgc tgttaattgt 720
gaaagccttc ataatagact tcgtttcttg caaaactctc ttggagcagt tccatctcct 780
attgattgtt acctctgcaa tcgaggtctg aagactctac atgtccgaat ggaaaagcat 840
ttcaaaaacg gaatggcagt tgcccagttc ctggaatcta atccttgggt agaaaagggt 900
atztatcctg ggctgccttc tcatccacag catgagttgg tgaagcgtca gtgtacagggt 960
tgtacaggga tggtcacctt ttatattaag ggcactcttc agcatgctga gattttcctc 1020
aagaacctaa agctatttac tctggccgag agcttgggag gattcgaaag ccttgctgag 1080
cttccggcaa tcatgactca tgcacagtt cttagaatg acagagatgt ccttgggaatt 1140
agtgcacac tgattcgact ttctgtgggc ttagaggatg aggaagacct actggaagat 1200
ctagatcaag ctttgaaggc agcacaccct ccaagtggaa ttcacagcta gtattccaga 1260
gctgtctatta gaagctgctt cctgtgaaga tcaatcttcc tgagtaatta atggaccaac 1320
aatgag                                     1326

```

<210> 29

<211> 49

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:PCR product

<400> 29

```

cccacggtcg gggtagcttg gcgggacgcg ccaggccgac tcccggcga 49

```

<210> 30

<211> 3464

<212> DNA

<213> Homo sapiens

<400> 30

```

tttaatggac acataattta attatatatt ttttcttaca gatacccagg tgttctctct 60
gatgtccagg aggagaaagg cattaagtac aaatttgaag tataatgagaa gaatgattaa 120
tatgaagggtg ttttctagtt taagttgttc cccctccctc tgaaaaaagt atgtattttt 180
acattagaaa aggttttttg ttgacttttag atctataatt atttctaagc aactagtttt 240
tattccccac tactcttgtc tctatcagat accatttatg agacattctt gctataacta 300
agtgtctctc caagacccca actgagtcct cagcacctgc tacagtgage tgccattcca 360
caccatcac atgtggcact cttgccagtc cttgacattg tcgggctttt cacatgttgg 420
taatatttat taaagatgaa gatccacata ccgttcaact gagcagtttc actagtggaa 480
ataccaaaag ctctctacgt gtatatccag aggtttgtag ataaatggtg ccacctgttt 540
tgtaacagtg aaaaattgaa aacaacctgg aagtccagtg atgggaaaat gagtatgttt 600
ctgtcttaga ttggggaacc caaagcagat tgcaagactg aaatttcagt gaaagcagtg 660
tatttgctag gtcataccag aaatcatcaa ttgaggtacg gagaaactga actgagaagg 720

```

```

taagaaaagc aatttaaagt cagcgagcag gttctcattg ataacaagct ccatactgct 780
gagatacagg gaaatggagg ggggaaagct ggagtattga tcccgcccc ctccttgggt 840
gtcagctccc tgctctgtgt gtgggcgga catagtccag ctgctctata gcaagtctca 900
ggtgttttgc gtaagaagct gctggcatgc acgggaacag tgaatgccaa acacttaaag 960
caattcgatg ttttaagtatg taagttcttt ttttttaga cagcgtttcg ctcttgttgc 1020
ccaggctagc atgcaatggt gtgacctcg cttactgcaa cctccgcctt ccagattca 1080
agcgattctc ctgcctcagg ctcccaagta gctaggacca ggtgcgcgcc accacgcccc 1140
gctaattttt gtattttgt ttttttagtag agatggggtt tcaccatggt ggtcaggcta 1200
gtctcgaact cgtgaccgca agcgattcac ccacctcagc ctcccaaagt gctgggatta 1260
ccggcttgag ccaccacacc cggcacatct tcattctttt tatgtagtaa aaagtataag 1320
gccacacatg gtttatttga agtattttat aatttaaaaa aatacagaag caggaaaacc 1380
aattataagt tcaagtgagg gatgatgggt gcttgaacca aagggttgca tgtagtaaga 1440
aattgtgatt taagatata tttaaagtta taagtagcag gatattctga tggagtttga 1500
ctttggtttt gggccagggt agtttcagat gcctttgaga aatgaatgaa gtagagagaa 1560
aataaaaagaa aaaccagcca ggcacagtgg ctcacacctg taatcccagc gctttgggag 1620
gctaaggcag gcagatcact tgagaccagc ttgggcaaca tggcaaagcc ccactctac 1680
aaaaaacaca aaaattagct gggcatttgt gcgcacacct gtattcccat ctagttagga 1740
agctgagatg gaagaattaa ttgagcccac gagttcaagg ctgcagttag tctgtattgt 1800
gccactgcac tccagccggg gtgacagaag agacctgtc tcgaaaacga atctgaaaac 1860
aatggaacca tgccctcata attctagaaa gttattttca actgataaat ctatattcac 1920
ccaaataatc aagggtgaag gtaaaataat acatttttag acaagcaaa actcaggggt 1980
tacctccatg tgcccttttt agggaagctg ttggagaaaa tactccagca aaatgaagga 2040
gtacacaaac cagagaatga catgaatcca gcaaatagga tccaacacag gcaatattcc 2100
agctatggag ctagctttaa aaaggaacag taaaaatatt aatcggttag ctgggtggaa 2160
tgggccatgc ctgtagtccc agctactcag gaggtcagc agcaggacga cttgagccca 2220
agagttccag accagcctgg ccaccttagt gagatccctt ctcttaaaaa taataactta 2280
ttgccagatt tggggcattt ggaaagaagt tcattgaaga taaagcaaaa gtaaaaaaaa 2340
aaaaaaaaaa aacaagggga aagggttggt taggcaatca ttctagggca gaaagaagta 2400
caggatagga agagcataat acactgtttt tctcaacaag gagcagtatg tacacagtca 2460
taatgatgtg actgcttagc ccctaaatat ggtaactact ctgggacaat atgggaggaa 2520
aagtgaagat tgtgatgggt taagagctaa tcctcatctg tcatatccag aaatcactat 2580
ataatatata ataataaat gactaagtta tgtgaggaaa aaaacagaag acattgctaa 2640
aagatgttaa agtcattgct ctggagaatt aggaggatg gggcagggga ctgttaggat 2700
gcattataaa ctgaaaagcc ttttttaaat tttatgtatt aatataatgca ttcacttgaa 2760
aaactaaaaa aaaacaataa tttggaaaaa ccatgaagg taactaacgg aaggaaaaac 2820
taagagaatg aaaagtattt gcctctggaa agaacaactg gcaggactgt tgttttcatt 2880
gtaagacttt tggagccatt taattgtact taaccatttt catctatttc ttaataaga 2940
acaattccat ctttaataaag agttacactt gttaataagt gctggcctcc tgttgttctt 3000
tgtacacccc acacaaaatt tcaaagaaac tttgatggca atatatctcc atgtcagct 3060
taaaaataga gaaaggaaaa catagaatta gccaaagatc acacaaaaca aagatcagtt 3120
gtttgttagg aaacaatcaa aatcaagtct cactttttcc agattggctt atggaacagc 3180
actgtaaggt gataacttgg ggcaaacatg taaataataa aacatatgtt ttaaatattc 3240
agggttagcac attttatgtt tctgtgagat taaaattgtg tgtgacatac ccgcttctt 3300
aaaggcaatg tttctgaaaa tgttgtaact gctatttctg aatcagggat ggggtccaga 3360
atctgccttt taaacatctc agataatctg aagcctgctt aagtttgtaa ggcactgctt 3420
ttgcactcta aggaagaaaa aaacaagttt taattcccg ctct 3464

```

<210> 31

<211> 1584

<212> DNA

<213> Homo sapiens

<400> 31

```

cggggcagct ctgaggaaca aggtggaagc tcagagcgct ggtctccacc ctggtgcccc 60
tggttggtg ctggcagtg gagccgtggc tgtggatgag agacatagac gagagagtga 120
gatggcctgg tttgccctct acctcctgag ccttctctgg gctacagctg ggactagtac 180
ccagaccag agttcatgct ccgttccctc agcacaggag cccttggtca atggaatata 240
agtactcatg gagaactcgg tgacttcac agcctacca aacccagca tcttgattgc 300
catgaatctg gccggagcct acaacttgaa ggcccagaag ctctgactt accagctcat 360
gtccagcgac aacaacgac taaccattgg gcacctcggc ctcaccatca tggccctcac 420
ctcctcctgc cgagaccctg gggataaaagt atccattcta caaagacaaa tggagaactg 480
ggcaccttcc agccccaacg ctgaagcatc agccttctat gggcccagtc tagcgatctt 540
ggcactgtgc cagaagaact ctgaggcgac cttgccgata gccgtccgct ttgccaagac 600
cctgctggcc aactcctctc ctttcaatgt agacacagga gcaatggcaa ccttggctct 660
gacctgtatg tacaacaaga tccctgtagg ttcagaggaa ggttacagat ccctggttgg 720
tcaggtaacta aaggatattg tggagaaaat cagcatgaag atcaaagata atggcatcat 780
tggagacatc tacagtactg gcctcgccat gcaggctctc tctgtaacac ctgagccatc 840
taaaaaggaa tggaaactgca agaagactac ggatatgata ctcaatgaga ttaagcaggg 900
gaaattccac aaccccatgt ccattgctca aatcctccct tccctgaaag gcaagacata 960
cctagatgtg ccccaggta cttgtagtcc tgatcatgag gtacaaccaa ctctacccag 1020
caaccctggc cctggcccca cctctgcac taacatcact gtcataata ccataataa 1080
ccagctgagg ggggttgagc tgetcttcaa cgagaccatc aatgttagtg tgaaaagtgg 1140
gtcagtgtta cttgtgtccc tagaggaagc acagcgcaa aatcctatgt tcaaatgtga 1200
aaccacaatg acatcttggg gccttgtcgt ccttctatc aacaatatcg cggaaaatgt 1260
taatcacaag acatactggc agtttcttag tgggtgaaca cctttgaatg aaggggttgc 1320
tgactacata cccttcaacc acgagcacat cacagccaat ttcacacagt actaacgaag 1380
aggtgggttc agcttctatc aaacatctcc aaaggatggg tgaaattttt tccacttcat 1440
tttaaatact tgcaaaaaag cgaatgcctg tgatgtacc atattcctgg taaaaacatg 1500
gagaaccact atgtagaata aaaatgcaaa gttcactgga gtctcaacat ctatgactca 1560
tgaaaataaa attttcatct tctc                                     1584

```

<210> 32

<211> 1537

<212> DNA

<213> Homo sapiens

<400> 32

```

gctctatta ccttctgccc atcacttaat aaatagccag ccaattcacc aacattctgg 60
tacactgttg gagagatgag acagtcacac cagctgcccc tagtggggct cttactgttt 120
tcttttattc caagccaact atgagagatt tgtgaggtaa gtgaagaaaa ctacatccgc 180
ctaaaacctc tgttgaatac aatgatccag tcaaaactata acaggggaac cagcgctgtc 240
aatgttgtgt tgtccctcaa acttgttgga atccagatcc aaaccctgat gcaaaagatg 300
atccaacaaa tcaaatataa tgtgaaaagc agattgtcag atgtaagctc gggagagctt 360
gccttgatta tactggcttt gggagtatgt cgtaacgctg aggaaaactt aatatatgat 420
taccacctga ctgacaagct agaaaataaa ttccaagcag aaattgaaaa tatggaagca 480
cacaatggca cccccctgac taactactac cagctcagcc tggacgtttt ggccttgtgt 540
ctgttcaatg ggaactactc aaccgcccga gttgtcaacc acttactccc tgaaaataaa 600
aactattatt ttggtagcca gttctcagta gatactgggt caatggctgt cctggctctg 660
acctgtgtga agaagagtct aataaatggg cagatcaaag cagatgaagg cagtttaaaag 720
aacatcagta tttatacaaa gtcactggta gaaaagattc tgtctgagaa aaaagaaaat 780
ggctcattg gaaacacatt tagcacagga gaagccatgc aggccctctt tgtatcatca 840
gactattata atgaaaatga ctggaattgc caacaaactc tgaatacagt gctcacggaa 900

```

```

atttctcaag gagcattcag taatccaaac gctgcagccc aggtcttacc tgccctgatg 960
ggaaagacct tcttggatat taacaaagac tcttcttgcg tctctgcttc aggttaacttc 1020
aacatctccg ctgatgagcc tataactgtg acacctctcg actcacaatc atatatctcc 1080
gtcaattact ctgtgagaat caatgaaaca tatttcacca atgtcactgt gctaaatggg 1140
tctgtcttcc tcagtgtgat ggagaaagcc cagaaaatga atgatactat atttggtttc 1200
acaatggagg agcgctcatg ggggccctat atcacctgta ttcagggcct atgtgccaac 1260
aataatgaca gaacctactg ggaacttctg agtggaggcg aaccactgag ccaaggagct 1320
ggtagttacg ttgtccgcaa tggagaaaac ttggagggttc gctggagcaa atactaataa 1380
gccc aaactt tcctcagctg cataaaatcc atttgcagtg gagttccatg tttattgtcc 1440
ttatgccttc ttcttcattt atcccagtac gagcaggaga gttaataacc tccccttctc 1500
tctctacatg ttcaataaaa gttgttgaaa gattaac 1537

```

<210> 33

<211> 1866

<212> DNA

<213> Homo sapiens

<400> 33

```

ccgattcttg ctcactgctc acccacctgc tgctgccatg aggcaccttg gggccttctc 60
cttctctctg ggggtccttg gggccctcac tgagatgtgt gaaataccag agatggacag 120
ccatctggta gagaagtgg gccagcacct ctacaccttg atggaccggc tttccctgga 180
gcacttgaac ccagcatct atgtgggect acgcctctcc agtctgcagg ctgggaccaa 240
ggaagacctc tacctgcaca gcctcaagct tggttaccag cagtgcctcc tagggctctg 300
cttcagcgag gatgacggtg actgccaggg caagccttcc atgggccagc tggccctcta 360
cctgctcgct ctcagagcca actgtgagtt tgcaggggc cacaaggggg acaggctggg 420
ctcacagctc aaatggttcc tggaggatga gaagagagcc attgggcatg atcacaaggg 480
ccacccccac actagctact accagtatgg cctgggcatt ctggccctgt gtctccacca 540
gaagcgggtc catgacagcg tgggtggaaa acttctgtat gctgtggaac ctttccacca 600
gggccaccat tctgtggaca cagcagccat ggcaggcttg gcattcacct gtctgaagcg 660
ctcaaacttc aacctggtc ggagacaacg gatcaccatg gccatcagaa cagtgcgaga 720
ggagatcttg aaggccaga cccccaggg ccactttggg aatgtctaca gcacccatt 780
ggcattacag ttctcatga cttcccccat gcctggggca gaactgggaa cagcatgtct 840
caaggcgagg gttgctttgc tggccagttc gcaggatgga gccttccaga atgctctcat 900
gatttccag ctgctgcccg ttctgaacca caagacctac attgatctga tcttccaga 960
ctgtctggca ccacagtc tgttgaacc agctgtgag accattctc agaccaaga 1020
gatcatcagt gtcacgctgc aggtgcttag tctcttgccg ccgtacagac agtccatctc 1080
tgttctggcc ggggtccaccg tgggaagatgt cctgaagaag gcccatgagt taggaggatt 1140
cacatatgaa acacaggcct cctcgtcagg cccctactta acctccgtga tggggaaagc 1200
ggccggagaa agggagtctt ggcagcttct ccgagacccc aacacccac tgttgcaagg 1260
tattgctgac tacagacca aggatggaga aaccattgag ctgaggctgg ttagctggt 1320
gccccgagc tccctcatcc cagcagcctc gcacactccc taggcttcta cctccctcc 1380
tgatgtccct ggaacaggaa ctcgcctgac cctgctgcca cctcctgtgc actttgagca 1440
atgccccctg ggatcacccc agccacaagc ctttcgaggg ccctatacca tggcccacct 1500
tggagcagag agccaagcat cttccctggg aagtctttct ggccaagtct ggccagcctg 1560
gccctgcagg tctcccatga aggccacccc atgggtctgat gggcatgaag catctcagac 1620
tccttggcaa aaaacggagt ccgcaggccg caggtgttgt gaagaccact cgttctgtgg 1680
ttggggctct gcaagaaggc ctcctcagcc cgggggctat ggccctgacc ccagctctcc 1740
actctgctgt tagagtggca gctctgagct ggttgtggca cagtagctgg ggagacctca 1800
gcagggtcgc tcagtgcctg cctctgacaa aattaaagca ttgatggcct gtggacctgc 1860
aaaaaa 1866

```

<210> 34
 <211> 2798
 <212> DNA
 <213> Homo sapiens

<400> 34
 gccctctccc acagcggagt ccaaaacagg cctaccagtc agttcttatt tctattgggt 60
 gtttccatgc tccaccatgt taagagctaa gaatcagctt tttttacttt caccctcatta 120
 cctgaggcag gtaaaagaat catcaggctc caggctcata cagcaacgac ttctacacca 180
 gcaacagccc cttcaccag aatgggctgc cctggctaaa aagcagctga aaggcaaaaa 240
 cccagaagac ctaatatggc acaccccgga agggatctct ataaaaccct tgtattccaa 300
 gagagatact atggacttac ctgaagaact tccaggagtg aagccattca cacgtggacc 360
 atatcctacc atgtatacct ttaggccttg gaccatccgc cagtatgctg gttttagtac 420
 tgtggaagaa agcaataagt tctataagga caacattaag gctggtcagc agggattatc 480
 agttgccttt gatctggcga cacatcgtgg ctatgattca gacaaccctc gagttcgtgg 540
 tgatgttggg atggctggag ttgctattga cactgtggaa gatacaaaaa ttctttttga 600
 tggaaatcct ttagaaaaaa tgtcagtttc catgactatg aatggagcag ttattccagt 660
 tcttgcaaat tttatagtaa ctggagaaga acaagggtga cctaaagaga aacttactgg 720
 taccatccaa aatgatatac taaaggaatt tatggttcga aatacatata tttttctccc 780
 agaaccatcc atgaaaatta ttgctgacat atttgaatat acagcaaagc acatgccaaa 840
 atttaattca atttcaatta gtggatacca tatgcaggaa gcaggggctg atgccattct 900
 ggagctggcc tatacttttag cagatggatt ggagtactct agaactggac tccaggctgg 960
 cctgacaatt gatgaatttg caccaagggt gtctttcttc tggggaattg gaatgaattt 1020
 ctatatggaa atagcaaaga tgagagctgg tagaagactc tgggctcact taatagagaa 1080
 aatgtttcag cctaaaaact caaaatctct tcttctaaga gcacactgtc agacatctgg 1140
 atggtcactt actgagcagg atccctacaa taatattgtc cgtactgcaa tagaagcaat 1200
 ggcagcagta tttggaggga ctcagctctt gcacacaaat tcttttgatg aagctttggg 1260
 tttgccaact gtgaaaagtg ctgcaattgc caggaacaca caaatcatca ttcaagaaga 1320
 atctgggatt cccaaagtgg ctgacccctg gggagggtct tacatgatgg aatgtctcac 1380
 aaatgatgtt tatgatgctg ctttaaagct cattaatgaa attgaagaaa tgggtggaat 1440
 ggccaaagct gtatgtgagg gaatacctaa acttcgaatt gaagaatgtg ctgcccgaag 1500
 acaagctaga atagattctg gttctgaagt aattgttggg gtaaataagt accagttgga 1560
 aaaagaagac gctgtagaag ttctggcaat tgataaact tcagtgcgaa acaggcagat 1620
 tgaaaaactt aagaagatca aatccagcag ggatcaagct ttggctgaac attgtcttgc 1680
 tgactaacc gaatgtgctg ctagcggaga tggaaatata ctggctcttg cagtggatgc 1740
 atctcgggca agatgtacag tgggagaaat cacagatgcc ctgaaaaagg tatttggtga 1800
 acataaagcg aatgatcgaa tgggtgagtgg agcatatcgc caggaatttg gagaaagtaa 1860
 agagataaca tctgctatca agagggttca taaattcatg gaacgtgaag gtcgcagacc 1920
 tcgtcttctt gtagcaaaaa tgggacaaga tggccatgac agaggagcaa aagttattgc 1980
 tacaggattt gctgatcttg gttttgatgt ggacataggc cctcttttcc agactcctcg 2040
 tgaagtggcc cagcaggctg tggatgcgga tgtgcatgct gtgggcgtaa gcaccctcgc 2100
 tgctggctcat aaaaccctag ttctgaact catcaaagaa cttaactccc ttggacggcc 2160
 agatattctt gtcattgtgtg gaggggtgat accacctcag gattatgaat ttctgtttga 2220
 agttgggtgt tccaatgtat ttggtcctgg gactcgaatt ccaaaggctg ccgttcagggt 2280
 gcttgatgat attgagaagt gtttggaata gaagcagcaa tctgtataat atcctctttt 2340
 tgttttagct tttgtctaaa atattatttt agttatgac aaagaagaga gtaaagctat 2400
 gtcttcaatt taatttcaat acctgatttg tactttcctt gaaagcttta ctttaaaaata 2460
 ccttacttat aggcctggtg tcatgctata agtatgtaca tacagtttca cttcaaaaat 2520
 aaaaaaaaaa ccctaaaaac tctctatact ctctataaca atactttatc aagaactctg 2580
 gacaatggta ttatttttaa aaatcatggg gatgtattta ttagaatgtt tcttataaat 2640
 ctctttcatt tttatattaa gaattaaact gtacctaaaa aaactctgac tattcccatt 2700

tctcagttta gcattacatt gtcttgagca ccagaaaata aaatccatat attaattaaa 2760
 acctatcttg aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa 2798

<210> 35
 <211> 1637
 <212> DNA
 <213> Homo sapiens

<400> 35
 aagaactggc ctgtacattt tcaaggaatt cttgagaggt tcttgagagag attctgggag 60
 ccaaactc cattgggac ctagctgttt tagagaacaa cttgtaatgg agccttcac 120
 tcttgagctg ccggctgaca cagtgcagcg cattgcggct gaactcaaat gccacccaac 180
 ggatgagagg gtggctctcc acctagatga ggaagataag ctgaggcact tcaggagtg 240
 cttttatatt cccaaaatac aggatctgcc tccagttgat ttatcattag tgaataaaga 300
 tgaaaatgcc atctatttct tgggaaattc tcttggcctt caaccaaaaa tgggttaaac 360
 atatcttgaa gaagaactag ataagtgggc caaaatagca gcctatgggc atgaagtggg 420
 gaagcgtcct tggattacag gagatgagag tattgtaggc cttatgaagg acattgtagg 480
 agccaatgag aaagaaatag ccctaataag tgctttgact gtaaatttac atcttctaata 540
 gttatcattt ttttaagccta cgccaaaacg atataaaatt cttctagaag ccaaagcctt 600
 cccttctgat cattatgcta ttgagtcaca actacaactt cacggactta acattgaaga 660
 aagtatgcgg atgataaagc caagagaggg ggaagaaacc ttaagaatag aggatattct 720
 tgaagtaatt gagaaggaag gagactcaat tgcagtgatc ctgttcagtg ggggtgcattt 780
 ttacactgga cagcacttta atattcctgc catcacaata gctggacaag cgaagggttg 840
 ttatgttggc tttgatctag cacatgcagt tggaaatggt gaactctact tacatgactg 900
 gggagtggat tttgcctgct ggtgttccta caagtattta aatgcaggag caggaggaat 960
 tgctggtgcc ttcattcatg aaaagcatgc ccatacgatt aaacctgcat tagtgggatg 1020
 gtttgggcat gaactcagca ccagatttaa gatggataac aaactgcagt taatccctgg 1080
 ggtctgtgga ttccgaattt caaatcctcc cattttgttg gtctgttctt tgcattgctag 1140
 ttttagagac ttttaagcaag cgacaatgaa ggcattgcgg aaaaaatctg ttttgctaac 1200
 tggctatctg gaatacctga tcaagcataa ctatggcaaa gataaagcag caaccaagaa 1260
 accagtgtg aacataatta ctccgtctca tgtagaggag cgggggtgcc agctaacaat 1320
 aacattttct gttccaaaca aagatgtttt ccaagaacta gaaaaaagag gagtgggttg 1380
 tgacaagcgg aatccaaatg gcattcgagt ggctccagt cctctctata attctttcca 1440
 tgatgtttat aaatttacca atctgtcac ttctatactt gactctgcag aaacaaaaaa 1500
 ttagcagtgt tttctagaac aacttaagca aattatactg aaagctgctg tggttatttc 1560
 agtattattc gatttttaatt tattgaaagt atgtcaccat tgaccacatg taactaacia 1620
 taaataatat accttac 1637

<210> 36
 <211> 1908
 <212> DNA
 <213> Homo sapiens

<400> 36
 gaattcatga aaacgtagct cgtcctcaaa aaaaacagaa gaggagtaat cattttaagg 60
 gagaaatata tacgaaagga acaagatttt gaagcaccca agctgccacc tacattaaaa 120
 cagggtaggt ggctaaacac cagtcttcaa tgcccttcca cagcctcagt ctgaaaaata 180
 ctgtgcaggt gacccaagtg aggggtcacc cttgggcttt tctgtggca gtatctctgg 240
 tttaaaaaca aacaaacgta cttattgcgt tgaaggacgg caacaggaag gactccatga 300
 ttagtcacat ctataccatc ctaagaaact ttatccacc aaactgtatt tcagacttta 360
 taatctaac tacaaaaagt gttcactggg gaactgcaca atatgactgc ttttaaccgt 420

```

agtgatttca aatattgagc catgctgttg cagtcttaaa aactggagac ctaagggcag 480
ctttcttcta gtcacccaat ccagcacttt tttaaaaaat cagtaaaact cttcgaccac 540
caaggaaaaa aaaaaaggat ggagggttaa agacgcaccc ctgcccaca agccccctca 600
tcagaatggg agtcaggaga cctgagttcc tgtctcaggc ctgccattaa aaacctgcat 660
aacctttgcc tatctcctca aacggaagta ctaaaacctc agcgcttcac ccaatttgta 720
gccccggctg ggctcttccc accttcccct tcttcagccc gccccttcct cctccagccc 780
tatcatcggg cggaggggtcc ccgcctccgc ccgccttacc cacaagcccc gcccccccag 840
ccccgatggc cctgcccagt cccagacaga acctactacg tgcggcggca gctggggcgg 900
gaaggcgggc gctgggggcg ctgcccgcgc tgcagcgcag ggtccacctg gtcggctgca 960
cctgtggagg aggaggtgga tttcaggctt cccgtagact ggaagaatcg gctcaaaacc 1020
gcttgccctc caggggctga gctggaggca gcgaggccgc ccgacgcagg cttccggcga 1080
gacatggcag ggcaaggatg gcagcccggc ggagggccc ggcgaggagc gcgaacccgc 1140
ggccgcagtt cccaggcgtc tgcgggcgcg agcacgcgcg gaccctgcgt gcgcccgggc 1200
ggggggggcg ggccctcgct gcacaaatag ggacgagggg gcggggcggc cacaatttcg 1260
cgccaaactt gaccgcgcgt tctgctgtaa cgagcgggct cggaggtcct cccgctgctg 1320
tcatggttgg ttcgctaaac tgcctcgctc ctgtgtccca gaacatgggc atcggcaaga 1380
acgggggacct gccctggcca ccgctcaggt atctgccggg ccggggcgat gggacccaaa 1440
cggggcgcagg ctgcccacgg tgcgggtacc tgggcgggac gcgcccggcg actcccggcg 1500
agaggatggg gccagacttg cggctctgcg tggcaggaag ggtgggccc actggattcc 1560
ccttttctgc tgcgcgggag gcccagttgc tgatttctgc ccggattctg ctgccgggtg 1620
aggtcttgcc ctgcggcgcc ctgcgccagg gcaaagtccc agccctggag aaaacacctc 1680
accctaccc acagcgtcc gtttgtcagg tgccttagag ctgcagccca agggataatg 1740
tttcgagtaa cgctgtttct ctaacttgta ggaatgaatt cagatatttc cagagaatga 1800
ccacaacctc ttcagtagaa ggtaatgtgg gattaagtag ggtcttgctt gatgaagttt 1860
accagtgcaa atgttagtta aatggaaagt tttccgtgtt aatctggg 1908

```

<210> 37

<211> 30

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:primer

<400> 37

cccacggctcg ggggtggccga ctcccggcga

30

<210> 38

<211> 21

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:primer

<400> 38

ctaaactgca tcgtcgctgt g

21

<210> 39

<211> 19

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:primer

<400> 39

aaaaggggaa tccagtcgg

19

<210> 40

<211> 19

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:PCR product

<400> 40

acctgggagg gacgcgcca

19

<210> 41

<211> 1275

<212> DNA

<213> Homo sapiens

<400> 41

```

ctgcagcgcc aggggtccacc tggctgggctg cacctgtgga ggaggagggtg gatttcaggc 60
ttcccgtaga ctggaagaat cggctcaaaa ccgcttgccct cgcaggggct gagctggagg 120
cagcgaggcc gcccgacgca ggcttccggc gagacatggc agggcaagga tggcagccc 180
gcggcagggc ccggcgagga gcgcgaaccc gcggccgcag tcccaggcg tctgcgggcg 240
cgagcacgcc gcgaccctgc gtgcgcgggg gcgggggggc ggggcctcgc ctgcacaaat 300
agggacgagg gggcgggggc gccacaattt cgcgccaaac ttgaccgcgc gttctgctgt 360
aacgagcggg ctcgagggtc ctcccgtctc tgtcatggtt ggttcgctaa actgcatcgt 420
cgctgtgtcc cagaacatgg gcatcggcaa gaacggggac ctgccctggc caccgctcag 480
gtatctgccg ggcggggggc atgggaccca aacgggcgca ggctgcccac ggtcggggta 540
cctgggcggg acgcgccagg ccgactcccg gcgagaggat ggggccagac ttgcgggtctg 600
cgctggcagg aagggtgggc ccgactggat tccccttttc tgctgcgcgg gaggcccagt 660
tgctgatttc tgcccggatt ctgctgcccg gtgaggtctt tgccctgcgg cgcctcgcgc 720
cagggcaaaag tcccagccct ggagaaaaca cctcaccctt acccacagcg ctccgtttgt 780
cagggtgcctt agagctcgag cccaagggat aatgtttcga gtaacgctgt ttctctaact 840
tgtaggaatg aattcagata tttccagaga atgaccacaa cctcttcagt agaaggtaat 900
gtgggattaa gtagggtctt gcttgatgaa gtttaccagt gcaaagtgtt gttaaatgga 960
aagttttccg tgtaaatctg ggaccttttc tcttattatg gatctgtatg atctgtatgc 1020
agtccccaaag gttcatttac cattattaaa aaatttttgt cttagaaatt ttatgtatgt 1080
caacgcacga gcaaattatc aggcattggg cagaattggc aactgggtgg aggcctcggc 1140
ggaggttagc actccgaaag gaaaacagag taggcctttg gaacagctgc tgggaagagat 1200
aaggcctgaa caagggcagt ggagaagaga gggtaaaaat tttttaaggt tacatgacct 1260
tgatttttgg agatc

```

1275

<210> 42

<211> 1256

<212> DNA

<213> Homo sapiens

<400> 42

```

ctgcagcgcc aggggtccacc tggctcggctg cacctgtgga ggaggaggtg gatttcaggc 60
ttcccgtaga ctggaagaat cggctcaaaa ccgcttgccct cgcaggggct gagctggagg 120
cagcgaggcc gcccgacgca ggcttccggc gagacatggc agggcaagga tggcagcccc 180
gcggcagggc ccggcgagga gcgcgaaccc gcggccgcag ttcccaggcg tctgcgggcg 240
cgagcacgcc gcgacctgc gtgcgcggg gcgggggggc ggggcctcgc ctgcacaaat 300
agggacgagg gggcggggcg gccacaattt cgcgccaaac ttgaccgcgc gttctgctgt 360
aacgagcggg ctcggaggtc ctcccgtgc tgtcatggtt ggttcgctaa actgcatcgt 420
cgctgtgtcc cagaacatgg gcatcggcaa gaacggggac ctgccctggc caccgctcag 480
gtatctgccc ggccggggcg atgggaccca aacgggcgca ggctgcccac ggtcggggtg 540
gccgactccc ggcgagagga tggggccaga cttgcggtct gcgctggcag gaagggtggg 600
cccgaactga ttcccctttt ctgctgcgcg ggaggcccag ttgctgattt ctgcccgat 660
tctgctgccc ggtgaggtct ttgccctgcg gcgccctcgc ccagggcaaa gtcccagccc 720
tggagaaaaa acctcacccc taccacagc gctccgtttg tcagggtcct tagagctcga 780
gccccaggga taatgtttcg agtaacgctg tttctctaac ttgtaggaat gaattcagat 840
atctccagag aatgaccaca acctcttcag tagaaggtaa tgtgggatta agtaggtct 900
tgcttgatga agtttaccag tgcaaatgtt agttaaatgg aaagttttcc gtgttaatct 960
gggacctttt ctcttattat ggatctgtat gatctgtatg cagttcccaa ggttcattta 1020
ccattattaa aaaatttttg tcttagaaat tttatgtatg tcaacgcacg agcaaattat 1080
caggcatggg gcagaattgg caactgggtg gaggtctcgg tggagggttag cactccgaaa 1140
ggaaaacaga gtaggccttt ggaacagctg ctggaagaga taaggcctga acaagggcag 1200
tggagaagag agggtaaaaa ttttttaagg ttacatgacc ctggattttg gagatc 1256

```

<210> 43

<211> 55

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:PCR product

<400> 43

```

gctgcccacg gtcggggtac ctgggcggga cgcgccaggc cgactccccg cgaga 55

```

<210> 44

<211> 36

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:PCR product

<400> 44

```

gctgcccacg gtcggggtgg ccgactcccc gcgaga 36

```

<210> 45

<211> 1273

<212> DNA

<213> Homo sapiens

<400> 45

```

ctgcagcgca ggggccacct ggctcggtgc acctgtggag gaggaggtgg atttcaggtc 60
tcccgtagac tggaagaatc ggctcaaaac cgcttgccct gcaggggctg agctggaggc 120
agcgaggccg cccgacgcag gcttccggcg agacatggca gggcaaggat ggagccccgg 180
cggcaggggc cggcgaggag cgcgaaacccg cggccgcagt tcccaggcgt ctgcggggcg 240
gagcacgccc cgaccctgcg tgcgccccgg cgggggggcg gggcctcgcc tgcacaaata 300
gggacgaggg ggcggggcgg ccacaatttc gcgccaaact tgaccgcgcg ttctgtgtga 360
acgagcgggg tcggaggtcc tcccgtgtgt gtcattggtg gttcgctaaa ctgcatcgtc 420
gctgtgtccc agaacatggg catcggaag aacggggacc tgccctggcc accgctcagg 480
tatctgccgg gccggggcga tgggacccaa acggggcgag gctgccacg gtcggggtag 540
ctgggcggga cgcgcgggac gactcccgcc gagaggatgg ggccagactt gcggtctgcg 600
ctggcaggaa ggggtggggc gactggattc cctttttctg ctgcgcggga ggcccagttg 660
ctgatttctg cccggattct gctgccccgt gaggtctttg cctgcggcg ccctcgccca 720
gggcaaagtc ccagccctgg agaaaacacc tcaccctac ccacagcgt ccgtttgtca 780
ggtgccttag agctcgagcc caagggataa tgtttcgagt aacgctgttt ctctaacttg 840
taggaatgaa ttcagatatt tccagagaat gaccacaacc tcttcagtag aaggtaatgt 900
gggattaagt aggggtcttg ttgatgaagt ttaccagtgc aaatgttagt taaatggaaa 960
gttttccgtg ttaatctggg accttttctc ttattatgga tctgtatgat ctgtatgcag 1020
ttcccaaggt tcatttacca ttattaaaaa atttttgtct tagaaatatt atgtatgtca 1080
acgcacgagc aaattatcag gcatggggca gaattggcaa ctgggtggag gcttcgggtg 1140
aggtagcac tccgaaagga aaacagagta ggcctttgga acagctgctg gaagagataa 1200
ggcctgaaca agggcagtg agaaagagag gtaaaaattt tttaaggtta catgaccctg 1260
gattttggag atc 1273

```

<210> 46

<211> 18

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:PCR product

<400> 46

acctgggcgg gacgcgcc

18

CORRECTED VERSION

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
30 November 2000 (30.11.2000)

PCT

(10) International Publication Number
WO 00/71754 A2

- (51) International Patent Classification: Not classified
- (21) International Application Number: PCT/US00/14354
- (22) International Filing Date: 24 May 2000 (24.05.2000)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
09/318,448 25 May 1999 (25.05.1999) US
- (63) Related by continuation (CON) or continuation-in-part (CIP) to earlier application:
US 09/318,448 (CON)
Filed on 25 May 1999 (25.05.1999)
- (71) Applicant (for all designated States except US): UNIVERSITY OF MEDICINE AND DENTISTRY OF NEW JERSEY [US/US]; Suite 3200, 335 George Street, P.O. Box 2688, New Brunswick, NJ 08903 (US).
- (72) Inventors; and
- (75) Inventors/Applicants (for US only): JOHNSON, William, G. [US/US]; 91 Stewart Road, Short Hills, NJ 07078 (US). STENROOS, Edward, Scott [US/US]; 2nd floor, 317 Ann Street, Harrison, NJ 07029 (US).
- (81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.
- (84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).
- Published:
-- Without international search report and to be republished upon receipt of that report.
- (48) Date of publication of this corrected version:
8 March 2001
- (15) Information about Correction:
see PCT Gazette No. 10/2001 of 8 March 2001, Section II
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 00/71754 A2

(54) Title: METHODS FOR DIAGNOSING, PREVENTING, AND TREATING DEVELOPMENTAL DISORDERS DUE TO A COMBINATION OF GENETIC AND ENVIRONMENTAL FACTORS

(57) Abstract: The present invention discloses a novel method for identifying an individual who may be susceptible to develop a developmental disorder. In one particular example, an individual is identified who is genetically susceptible to becoming schizophrenic. In addition, the present invention discloses a novel method for identifying individuals who are genetically susceptible to have offspring with a developmental disorder. Methods of diagnosing, preventing and treating developmental disorders such as schizophrenia are also provided.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
30 November 2000 (30.11.2000)

PCT

(10) International Publication Number
WO 00/71754 A3

- (51) International Patent Classification⁷: C12Q 1/68, (74) Agent: DAVIS, Michael, D.; Klauber & Jackson, 411
C07K 14/47, C12N 15/85 Hackensack Avenue, Hackensack, NJ 07601 (US).
- (21) International Application Number: PCT/US00/14354 (81) Designated States (*national*): AE, AG, AL, AM, AT, AU,
AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE,
(22) International Filing Date: 24 May 2000 (24.05.2000) DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU,
ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS,
(25) Filing Language: English LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO,
NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR,
TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.
- (26) Publication Language: English (84) Designated States (*regional*): ARIPO patent (GH, GM,
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian
(30) Priority Data: 09/318,448 25 May 1999 (25.05.1999) US patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European
patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE,
(63) Related by continuation (CON) or continuation-in-part (CIP) to earlier application: IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG,
CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).
- US 09/318,448 (CON)
Filed on 25 May 1999 (25.05.1999) Published:
— with international search report
- (71) Applicant (*for all designated States except US*): UNI- (88) Date of publication of the international search report:
VERSITY OF MEDICINE AND DENTISTRY OF 28 March 2002
NEW JERSEY [US/US]; Suite 3200, 335 George Street,
P.O. Box 2688, New Brunswick, NJ 08903 (US).
- (72) Inventors; and (15) Information about Correction:
Previous Correction:
(75) Inventors/Applicants (*for US only*): JOHNSON, see PCT Gazette No. 10/2001 of 8 March 2001, Section II
William, G. [US/US]; 91 Stewart Road, Short Hills, NJ
07078 (US). STENROOS, Edward, Scott [US/US]; 2nd
floor, 317 Ann Street, Harrison, NJ 07029 (US).
For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 00/71754 A3

(54) Title: METHODS FOR DIAGNOSING, PREVENTING, AND TREATING DEVELOPMENTAL DISORDERS DUE TO A COMBINATION OF GENETIC AND ENVIRONMENTAL FACTORS

(57) Abstract: The present invention discloses a novel method for identifying an individual who may be susceptible to develop a developmental disorder. In one particular example, an individual is identified who is genetically susceptible to becoming schizophrenic. In addition, the present invention discloses a novel method for identifying individuals who are genetically susceptible to have offspring with a developmental disorder. Methods of diagnosing, preventing and treating developmental disorders such as schizophrenia are also provided.

INTERNATIONAL SEARCH REPORT

International Application No

PC 1/US 00/14354

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12Q1/68 C07K14/47 C12N15/85

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, SEQUENCE SEARCH, MEDLINE, BIOSIS, EMBL

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>PAULING LINUS: "Orthomolecular psychiatry: Varying the concentrations of substances normally present in human body may control mental disease (Originally published in Science, Volume 160, Pages 265-271, April 19, 1968)."</p> <p>JOURNAL OF NUTRITIONAL & ENVIRONMENTAL MEDICINE (ABINGDON),</p> <p>vol. 5, no. 2, 1995, pages 187-198,</p> <p>XP001031212</p> <p>ISSN: 1359-0847</p> <p>page 187</p> <p>page 193-197</p> <p style="text-align: center;">---</p> <p style="text-align: center;">-/--</p>	1-29

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *A* document member of the same patent family

Date of the actual completion of the international search

22 November 2001

Date of mailing of the international search report

05/12/2001

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl.
Fax: (+31-70) 340-3016

Authorized officer

Reuter, U

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 00/14354

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
X	REGLAND B ET AL: "Homozygous thermolabile methylenetetrahydrofolate reductase in schizophrenia-like psychosis." JOURNAL OF NEURAL TRANSMISSION, vol. 104, no. 8-9, 1997, pages 931-941, XP001031228 the whole document ---	1-29
X	ARINAMI TADAO ET AL: "Methylenetetrahydrofolate reductase variant and schizophrenia/depression." AMERICAN JOURNAL OF MEDICAL GENETICS, vol. 74, no. 5, 1997, pages 526-528, XP001031351 ISSN: 0148-7299 the whole document ---	1,5-14, 19-23
X	BROWN ALAN S ET AL: "Neurobiological plausibility of prenatal nutritional deprivation as a risk factor for schizophrenia." JOURNAL OF NERVOUS AND MENTAL DISEASE, vol. 184, no. 2, 1996, pages 71-85, XP001031380 ISSN: 0022-3018 page 73 page 80-83 ---	4,24-27, 29
X	LEWIS DALE P ET AL: "Drug and environmental factors associated with adverse pregnancy outcomes. Part III: Folic acid: Pharmacology, therapeutic recommendations and economics." ANNALS OF PHARMACOTHERAPY, vol. 32, no. 10, October 1998 (1998-10), pages 1087-1095, XP001031383 ISSN: 1060-0280 the whole document ---	1,4-14, 19-27,29
X	DATABASE EMBL 'Online! Acc. nb. AA744384, 19 January 1998 (1998-01-19) "Homo sapiens cDNA clone IMAGE:1282963 3' similar to gb:J00140 Dihydrofolate reductase (human)" XP002183710 abstract ---	39-41
X	WO 99 01560 A (BOWTELL DAVID ;KILIAN ANDRZEJ (AU); CAMBIA BIOSYSTEMS LLC (US)) 14 January 1999 (1999-01-14) page 41, column 35 ---	39,42,43

-/--

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/US 00/14354

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 90 02203 A (SCANLON KEVIN J) 8 March 1990 (1990-03-08) page 20, line 9 ---	33-36
X	CHEN M-J ET AL: "THE FUNCTIONAL HUMAN DI HYDRO FOLATE REDUCTASE EC-1.5.1.3 GENE" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 259, no. 6, 1984, pages 3933-3943, XP002183708 ISSN: 0021-9258 the whole document ---	33-39, 42, 43
A	NAURATH HANS J ET AL: "Effects of vitamin B12, folate, and vitamin B6 supplements in elderly people with normal serum vitamin concentrations." LANCET (NORTH AMERICAN EDITION), vol. 346, no. 8967, 1995, pages 85-89, XP002183709 ISSN: 0099-5355 the whole document ---	1-29
P, X	WO 00 04194 A (VARIAGENICS INC) 27 January 2000 (2000-01-27) claims -----	1, 5-14, 19-23

INTERNATIONAL SEARCH REPORT

information on patent family members

International Application No

PCT/US 00/14354

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9901560	A	14-01-1999	
		AU 8285498 A	25-01-1999
		CN 1270634 T	18-10-2000
		EP 0917579 A1	26-05-1999
		WO 9901560 A1	14-01-1999
WO 9002203	A	08-03-1990	
		US 5085983 A	04-02-1992
		AU 633271 B2	28-01-1993
		AU 4197689 A	23-03-1990
		DE 68928500 D1	29-01-1998
		DE 68928500 T2	09-07-1998
		EP 0408675 A1	23-01-1991
		EP 0732409 A2	18-09-1996
		JP 10313880 A	02-12-1998
		JP 4500458 T	30-01-1992
		WO 9002203 A1	08-03-1990
		US 5618702 A	08-04-1997
		US 5736326 A	07-04-1998
		US 5585363 A	17-12-1996
		US 5166140 A	24-11-1992
		US 5814489 A	29-09-1998
		CA 2016667 A1	17-11-1990
		NZ 233660 A	23-12-1992
WO 0004194	A	27-01-2000	
		AU 5116899 A	07-02-2000
		EP 1100964 A1	23-05-2001
		WO 0004194 A1	27-01-2000

Form PCT/ISA/210 (patent family annex) (July 1992)